# The Basement Membrane in the Human Diabetic Eye

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There is increasing evidence that the pathologic changes that characterize the vascular complications of diabetes involve thickening and hyalinization of basement membranes.[1,2] The present study confirms previous findings in the retinal capillaries and demonstrates the remarkable thickening of the basement membrane of the ciliary processes in those eyes with diabetic retinopathy.

## MATERIALS AND METHODS

The eyes of thirty-nine diabetic patients and 175 nondiabetics were obtained at autopsy. On the basis of celloidin sections and flat preparations of the retina stained with periodic acid Schiff (PAS), twenty-seven of the eyes from diabetics were classified as showing retinopathy. No retinal lesions of a diabetic type were found in the remaining twelve eyes. The kidneys of the same diabetic patients had been studied and classified independently as to the presence of Kimmelstiel-Wilson lesions on the basis of random sections.

Using 14 to 20$\mu$ celloidin sections, the ciliary processes of all normal and diabetic eyes were studied and photographed along with a scaled slide. The thickness of the basement membrane of the ciliary processes was measured on photographic enlargements averaging values from various parts of the ciliary processes. The results of these measurements were compared for different age groups in the nondiabetic population and for the presence or absence of retinopathy in the diabetic group. In addition the pathology of the retinal capillary aneurysms and the alterations that constitute diabetic iridopathy were reviewed.

## RESULTS

1. *Basement membrane of the ciliary processes.*

A. *Age changes.* The thickness of the basement membrane of the ciliary processes was found to increase with age from mean values of less than 1$\mu$ under the age of ten years to 4$\mu$ at age forty years and values over 4.5$\mu$ after age sixty (table 1, figures 1, 2, and 3).

TABLE 1

Basement membrane of ciliary processes in eyes of nondiabetics

| Age | Number of patients | Thickness basement membrane micra (mean±S.D.) |
|---|---|---|
| Under 5 years | 23 | Not measurable |
| 5 - 9 | 10 | 0.8±0.4 |
| 10 - 19 | 8 | 2.1±0.7 |
| 20 - 39 | 23 | 3.4±0.8 |
| 40 - 59 | 61 | 4.0±1.1 |
| 60 and over | 50 | 4.7±1.0 |

B. *Diabetes mellitus.* In diabetics with retinopathy a markedly thickened basement membrane was demonstrated (tables 2 and 3, figures 1 and 4). The mean value for the entire retinopathy group was 10.5$\mu$±1.8 (S.D.) as compared with an average value of 4.1$\mu$ for the nondiabetics of the same ages. In addition to this highly significant difference, there was no variation in the diabetic retinopathy group of basement membrane thickness with age: averaging 11.8$\mu$ ± 2.1$\mu$ under the age of forty, 10.2$\mu$ ± 1.1$\mu$ between forty and fifty-nine

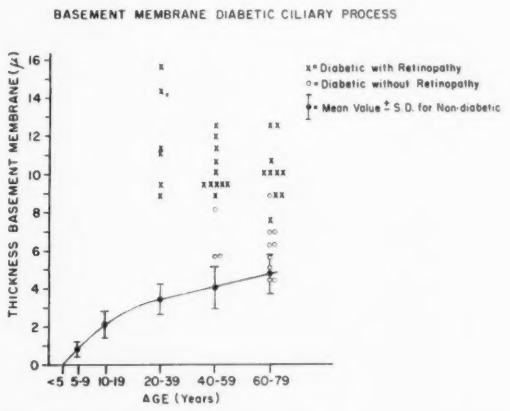


FIG. 1. The variation with age of the thickness of the basement membrane of the human ciliary processes of diabetic and nondiabetic eyes.

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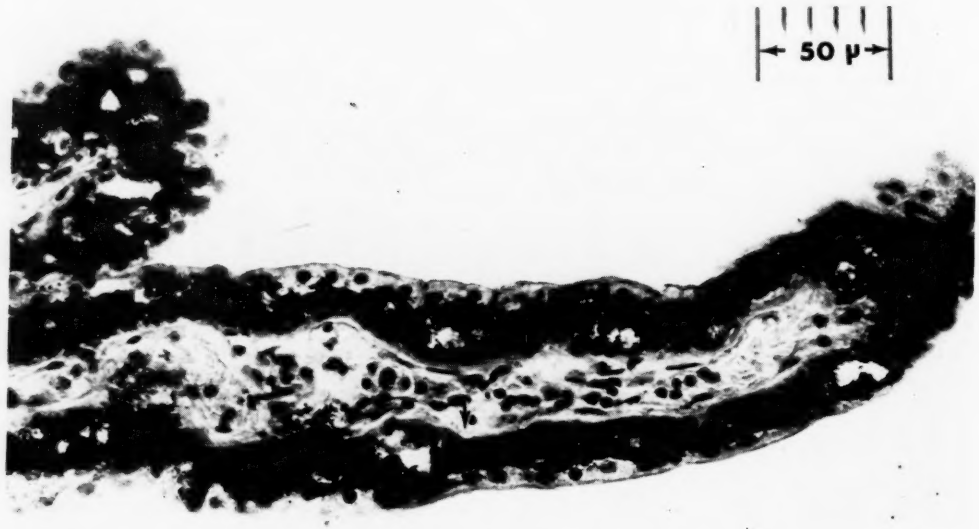


FIG. 2. Basement membrane (↓) of ciliary process of normal eye obtained at autopsy from seven-year-old colored male measured from 0.2$\mu$ to 1.9$\mu$ (average 1.1$\mu$). PAS, hematoxylin, X 250.

FIG. 3. Basement membrane (↑) of ciliary process of nondiabetic eye obtained at autopsy from sixty-one-year-old white female, measured from 2.5$\mu$ to 7.5$\mu$ (average 5.0$\mu$). PAS, hematoxylin, X 250.

FIG. 4. Basement membrane (↓) of ciliary process of eye with diabetic retinopathy and nephropathy from sixty-one-year-old white female, who has had twenty-four-year history of diabetes, measured from 7.5μ to 12.5μ (average 10.0μ). Basement membrane stained very deeply with PAS. PAS, hematoxylin, X 250.

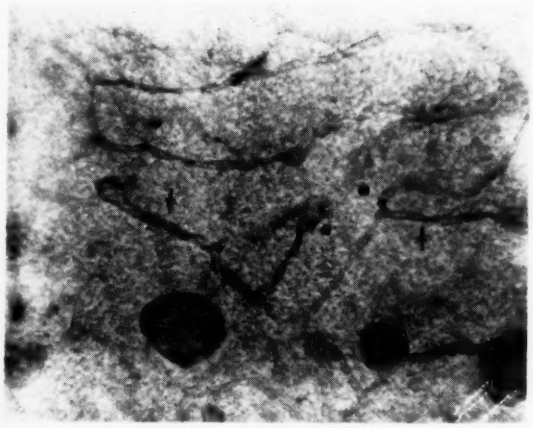

FIG. 5. Flat preparation of hyalinized, irregular and partly obliterated capillaries at venous side of aneurysm (↓↑). From seventy-one-year-old white female with thirty-year history of diabetes. PAS, X 207.

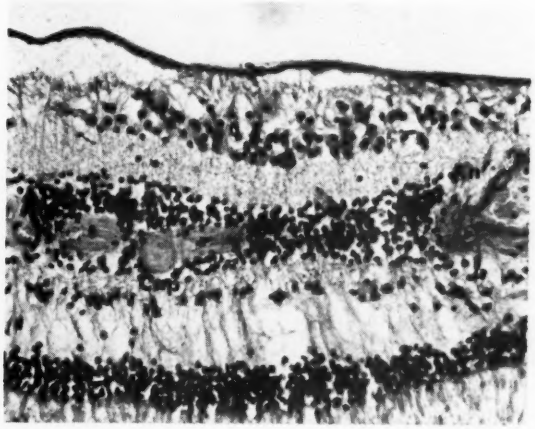

FIG. 6. Cross section of same area of the eye of figure 5. Capillaries (Cap) adjacent to aneurysm (An) have markedly thickened PAS positive walls and are partially obliterated. PAS, hematoxylin, X 207.

FIG. 7. Cross section of capillary aneurysm with thick PAS staining hyalinized wall in bipolar cell layer, extending into external plexiform layer. The wall appears to be continuous with the basement membrane of the adjacent capillary. The inside of the wall is lined with endothelium with a small lumen in the center. From sixty-nine-year-old colored female with two-year history of poorly controlled diabetes and with Kimmelstiel-Wilson disease. PAS, hematoxylin, X 443.

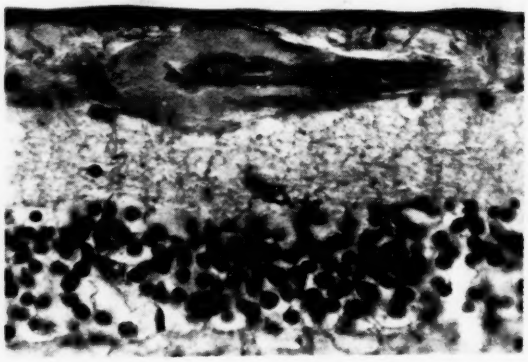

FIG. 9. Cross section of same area as figure 8, demonstrating venule with markedly thickened PAS positive wall and very narrowed or partly obliterated lumen in the superficial layer of the retina. PAS, hematoxylin, X 372.

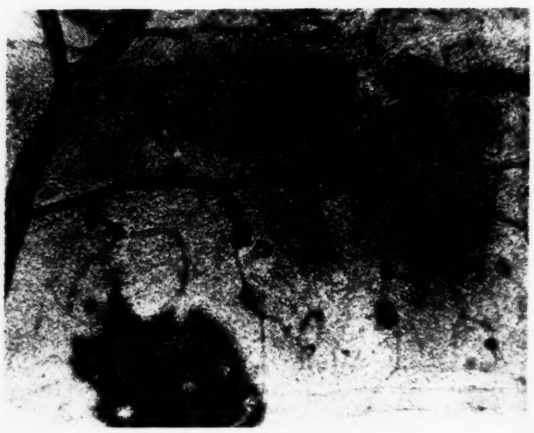

FIG. 10. Aneurysms developing at capillaries adjacent to precapillary arteriole (Art) or in precapillary arteriole itself. From sixty-year-old white female with twenty-four-year history of diabetes. PAS, X 83.

FIG. 8. Flat preparation of hyalinized and irregular venule (↓) connecting with capillary from aneurysm (An). From thirty-three-year-old white female with twenty-seven-year history of diabetes. PAS, X 207.

TABLE 2

Basement membrane of ciliary process in diabetes mellitus

| Patient | Age | Race | Sex | Years diabetes (? = unknown) | Retinopathy (+ = present; — = absent) | Iridopathy | Nephropathy | Thickness BM(μ) |
|---|---|---|---|---|---|---|---|---|
| 1 | 29 | W | M | 24 | + | + | + | 15.6 |
| 2 | 33 | W | F | 27 | + | + | + | 14.4 |
| 3 | 33 | W | M | 20 | + | — | + | 8.8 |
| 4 | 37 | W | M | 23 | + | + | + | 11.3 |
| 5 | 37 | W | M | 30 | + | + | + | 9.4 |
| 6 | 38 | W | F | 28 | + | + | + | 11.3 |
| 7 | 44 | C | F | 5 | + | — | ? | 9.4 |
| 8 | 46 | C | M | 15 | + | + | + | 9.4 |
| 9 | 46 | W | M | ? | — | — | — | 5.6 |
| 10 | 49 | W | M | 11 | — | + | — | 8.1 |
| 11 | 49 | W | M | 22 | + | — | + | 10.6 |
| 12 | 49 | W | F | 20 | + | + | + | 9.4 |
| 13 | 53 | W | F | 25 | + | — | + | 11.9 |
| 14 | 55 | C | F | 19 | + | + | + | 10.0 |
| 15 | 56 | C | M | 23 | + | — | + | 9.4 |
| 16 | 56 | W | F | 2 | — | — | — | 5.6 |
| 17 | 57 | C | M | ? | + | — | + | 8.8 |
| 18 | 57 | C | M | 25 | + | + | + | 9.4 |
| 19 | 58 | W | F | ? | + | + | + | 12.5 |
| 20 | 59 | W | F | 20 | + | + | + | 11.3 |
| 21 | 60 | W | M | 7 | — | — | — | 6.9 |
| 22 | 60 | C | M | ? | + | — | + | 10.0 |
| 23 | 60 | C | F | 20 | + | + | + | 7.5 |
| 24 | 61 | W | F | 24 | + | + | + | 10.0 |
| 25 | 61 | C | F | ? | + | + | + | 12.5 |
| 26 | 62 | W | M | ? | — | — | — | 5.0 |
| 27 | 66 | C | F | 11 | — | — | — | 4.4 |
| 28 | 66 | W | F | 7 | — | — | — | 4.4 |
| 29 | 69 | W | F | 20 | + | + | + | 12.5 |
| 30 | 69 | C | F | 2 | + | + | + | 10.0 |
| 31 | 69 | W | M | 6 | — | + | — | 8.8 |
| 32 | 70 | C | F | ? | — | — | — | 5.6 |
| 33 | 71 | W | F | 30 | + | + | + | 10.0 |
| 34 | 71 | W | F | 8 | + | + | — | 10.6 |
| 35 | 73 | W | F | ? | — | — | — | 6.2 |
| 36 | 73 | W | F | 12 | + | + | — | 8.8 |
| 37 | 75 | W | M | 3 | — | — | — | 6.2 |
| 38 | 80 | C | M | ? | — | — | — | 6.9 |
| 39 | 84 | W | F | 10 | + | + | + | 8.8 |

TABLE 3

Basement membrane of ciliary processes in eyes of diabetics

A. DIABETICS WITH RETINOPATHY

| Age (years) | Number of patients | Thickness basement membrane micra (mean±S.D.) |
|---|---|---|
| 20 - 39 | 6 | 11.8±2.1 |
| 40 - 59 | 11 | 10.2±1.1 |
| 60 and over | 10 | 10.1±1.5 |
| Total | 27 | 10.5±1.8 |

B. DIABETICS WITHOUT RETINOPATHY

| | | |
|---|---|---|
| 40-59 | 3 | 6.4±1.2 |
| 60 and over | 9 | 6.0±1.1 |
| Total | 12 | 6.1±1.1 |

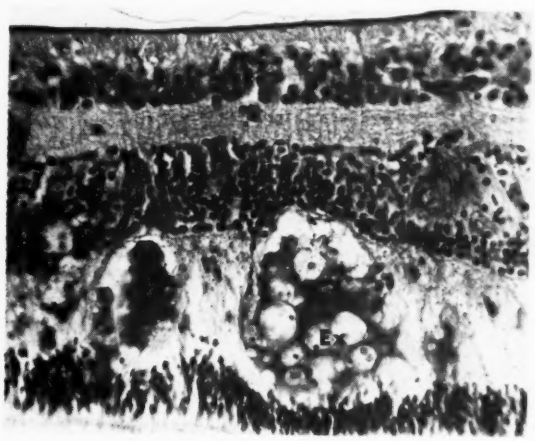


FIG. 11. Deep exudate (Ex) at outer plexiform layer, deeply stained with PAS and containing "phagocyte"-like cells. From sixty-one-year-old white female with twenty-four-year history of diabetes. PAS, hematoxylin, X 317.

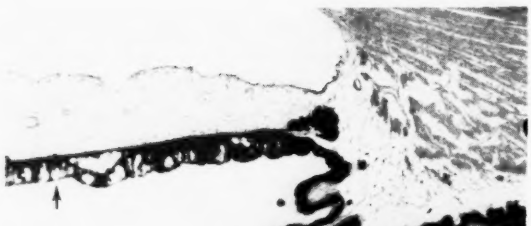

FIG. 12. Vacuolar degeneration of iris pigment epithelium (↑) and thickening of ciliary basement membrane (↓). From thirty-eight-year-old white female with twenty-eight-year history of diabetes. PAS, hematoxylin, X 83.

years, and 10.1$\mu$ ± 1.5$\mu$ for ages sixty and over. The basement membrane of the ciliary processes in the eyes with diabetic retinopathy stained a deeper and more purple color with PAS as compared with nondiabetic eyes, suggesting possible qualitative as well as quantitative changes.

Diabetic nephropathy was found on routine sections in twenty-four of the twenty-seven patients with retinopathy, and twenty of the retinopathy group had iridopathy in the same eyes. No differences in thickness of the basement membrane of the ciliary processes were noted between those retinopaths with and those without nephropathy or iridopathy.

In those eyes from diabetics without evidence of retinopathy, the basement membrane of the ciliary processes had a mean thickness (6.1$\mu$ ± 1.1$\mu$ S.D.) which was greater than that for the normal population of the same age group but much less than the retinopathy group. However, there was overlap in the nonretinopathy series with both the nondiabetic and the diabetic with

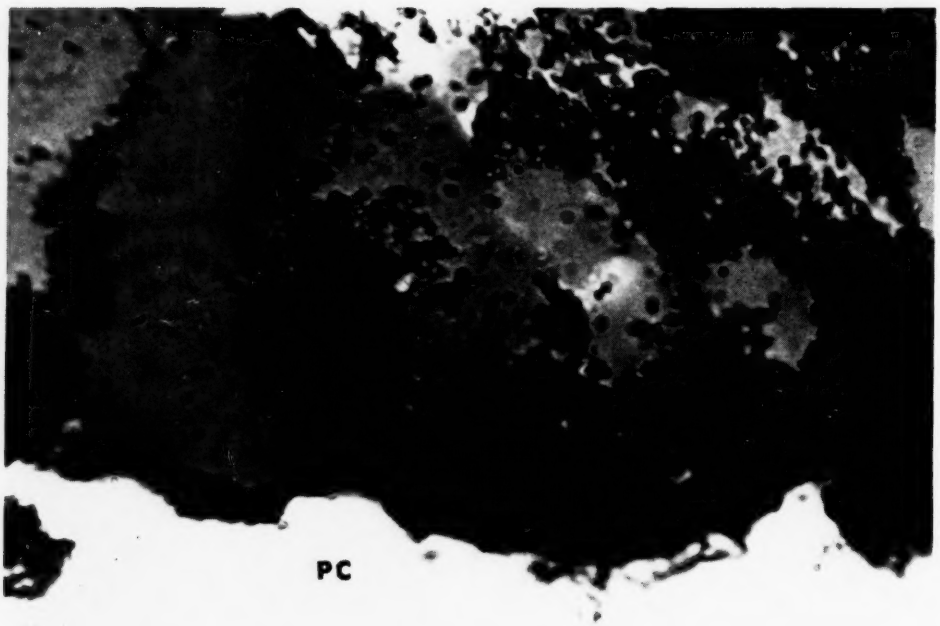


FIG. 13. PAS-positive material filling the vacuoles (Vac) of the iris pigment epithelium. From sixteen-year-old white male with eleven-year history of diabetes and retinopathy, rubeosis iridis and glaucoma. PC = posterior chamber—osmic acid fixation, methacrylate embedded. One micron section, PAS (oil immersion).

retinopathy. It is of interest that the two eyes in the group which had iridopathy had significantly higher values (8.1$\mu$ and 8.8$\mu$) than did the rest of the non-retinopathy group. None of the twelve patients in this classification had evidence of diabetic nephropathy.

2. *Retinal capillary aneurysms.*

In the course of these studies it was possible to demonstrate patent and hyalinized retinal capillary aneurysms both on flat preparation and cross section. On careful inspection the PAS-positive wall of capillary aneurysms appeared to be continuous with the basement membrane of the capillaries (figure 7). The venous capillaries adjacent to the aneurysms were often hyalinized, narrow, and irregular (figures 5, 6, 8, 9). Although aneurysms commonly develop toward the venous side of the capillaries, they were found in all portions of the capillaries, even immediately adjacent to precapillary arterioles (figure 10). Deep exudates in the outer plexiform layer were brilliantly stained with PAS and occasionally contained swollen phagocytic cells (figure 11).

3. *Diabetic iridopathy.*

Diabetic iridopathy was present in twenty of the twenty-seven eyes with retinopathy and two of the twelve eyes without retinopathy. All twenty-two eyes with diabetic iridopathy had markedly thickened basement membranes of the ciliary processes (mean values of 10.5$\mu$ $\pm$ 2.0$\mu$ S.D.).

Vacuoles of the pigment epithelium of the iris were filled with material which stained intensely with PAS, especially when they were fixed promptly in osmic acid and embedded in methacrylate (figures 12 and 13). The PAS positive material could be removed by incubation with saliva prior to staining (figure 14).

## DISCUSSION

An increase with age in the thickness of the basement membrane of the ciliary process is similar to that described in the rat kidney.[3] The role of this thickening in the aging process and, in particular, in the metabolism of the lens, vitreous, iris epithelium, and retina is of considerable interest. The marked changes in the basement membrane in the diabetic correlate well with the presence of retinopathy, iridopathy and nephropathy.

Its effect on aqueous humor dynamics and the

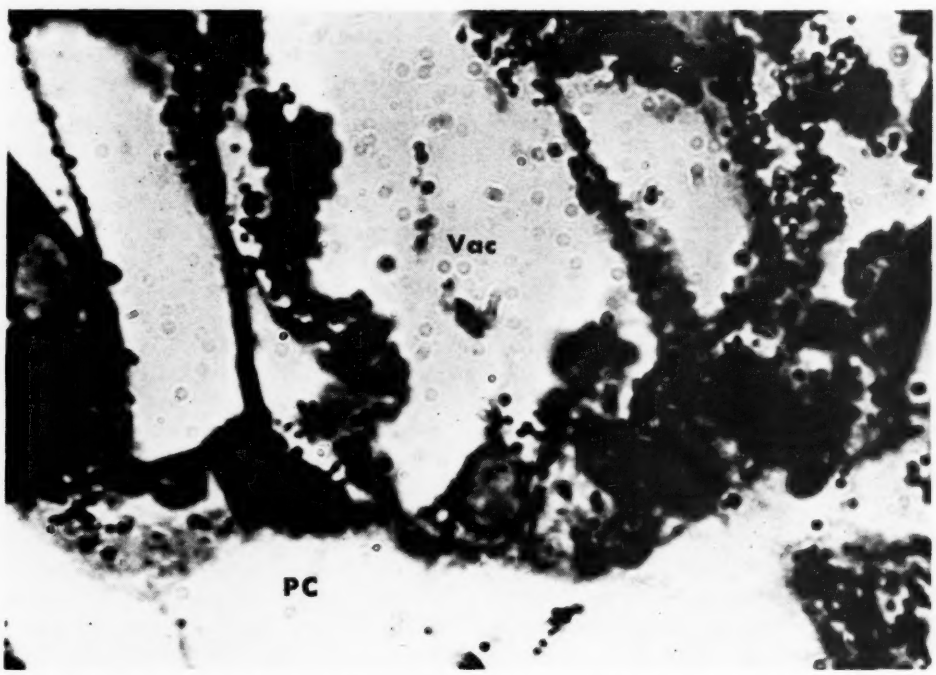


FIG. 14. Adjacent section of figure 13 treated with saliva for two hours prior to PAS staining. PAS staining material in the vacuoles (Vac) was completely removed. PC = posterior chamber osmic acid fixation, methacrylate embedded. One micron section, PAS (oil immersion).

nutrition of the lens, vitreous, and retina remains speculative.

In both the retinopathy and the nonretinopathy groups no correlation could be found between the width of the basement membrane of the ciliary processes and the length of time the patient had diabetes. However, as anticipated, the retinopathy group had diabetes of much longer duration than the nonretinopathy group.

The findings in the retinal capillary aneurysms strongly suggest a thickening of the basement membrane not only of the aneurysms themselves, but of adjacent capillaries and venules. The possible role of changes in small veins in the development of the capillary aneurysms remains speculative.

The present studies confirm the suspicion that glycogen or glycogen-like polysaccharides are major components of the PAS-positive material in the vacuoles of the iris epithelium in diabetic iridopathy.


## SUMMARY

1. The basement membrane of the ciliary processes of human eyes was found to increase in thickness progressively with age from less than $1\mu$ below age ten years to $4\mu$ or more beyond age forty years.

2. Diabetics with retinopathy, nephropathy and iridopathy demonstrated a remarkable thickening of the basement membrane of the ciliary processes at all ages. The mean value ($\pm$S.D.) for diabetics with retinopathy ($10.5\mu \pm 1.8$) was significantly increased over the nondiabetic of comparable age ($4.1\mu \pm 1.2$) and the diabetic without retinopathy ($6.1\mu \pm 1.1$).

## SUMMARIO IN INTERLINGUA

*Le Membrana Basal in le Oculo del Diabetico Human*

1. Esseva constatate que le membrana basal del processos ciliari in le oculo human cresce progressivemente in spissor con le etate, mesurante minus que $1\mu$ a etates de minus que 10 annos e $4\mu$ o plus a etates de 40 annos e plus.

2. Diabeticos con retinopathia, nephropathia, e iridopathia monstrava un remarcabile spissification del membrana basal del processos ciliari a omne etates. Le valor medie ($\pm$ D.S.) pro diabeticos con retinopathia ($10{,}5\mu \pm 1{,}8$) esseva significativemente augmentate in comparation con non-diabeticos de simile etates ($4{,}1\mu \pm 1{,}2$) e con diabeticos sin retinopathia ($6{,}1\mu \pm 1{,}1$).



## ACKNOWLEDGMENT

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# *Effect of Carbohydrate Source on Cholesterol and Cardiovascular System*

In suckling rabbits, levels of serum cholesterol are elevated (J. H. Bragdon, *Circulation* 5:641, 1952), and chickens fed sucrose-cholesterol diets develop higher cholesterol levels in both sera and livers than do chickens fed glucose-cholesterol food mixtures (D. Kritchevsky, et al. *Circulation* 20:964, 1959). There are old reports of high incidences of atherosclerosis in certain primitive peoples subsisting on diets containing large amounts of milk (B. Kuczynski, *Klin. Wchnschr.* 4:39, 1925). Many of our own American population, while not primitive, should also be regarded as heavy milk drinkers. There is a recent report, based on autopsy data, that the incidence of coronary arterial thrombosis and myocardial infarction in patients with peptic ulcers treated with Sippy diets was twice that found in a group of selected control subjects with peptic ulcers not subjected to this therapy (W. A. Thomas et al. *Circulation* 20:992, 1959).

Whether or not such random observations in man can be related to the experiments of Wells and Anderson cannot be decided now. However, there are sufficient data at hand to warrant active pursuit of these leads in future experimental and epidemiologic attacks on cardiovascular disease. It would be of interest to know whether intakes of animal carbohydrate parallel those of saturated fats in populations with high or low susceptibilities to myocardial infarction. There should be a search for other nutrients related to heart disease in addition to those associated with fats and sterols.

From *Nutrition Reviews,* Vol. 18, No. 3, pp. 89-90, March 1960.

# The Genetics of Diabetes Mellitus

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On reading the literature dealing with diabetes, everyone must be struck by the lack of precise knowledge which exists concerning the method of inheritance of the disorder, and in such a situation it is almost certain that what *is* known will provide the ingredients for heated controversy.

"Oh never, never let us doubt,
The things we are not sure about."

However, if we think about the matter it is perhaps not surprising that confusion exists because diabetes is such a heterogeneous condition that different investigators may well be dealing with predominantly different types of the disease. For example, some will be investigating it in Scots, others in Englishmen, and others still in Jamaicans and Kampalians. There will, therefore, be both racial and dietetic differences to take into account. Then there are fat diabetics and thin diabetics, late-onset diabetics and early-onset diabetics, those fertile and those barren, and those with other conditions such as acromegaly, thyrotoxicosis, hemachromatosis and "stress" syndromes such as burns, and patients with coronary thrombosis. In this list some are insulin-sensitive and some resistant, some give a clear-cut family history, while in some it seems doubtful whether there is an inherited component at all—gluttony or steroids may be the only factors.

What is generally agreed by all authorities is that sibs of those affected are themselves more often diabetic than are the sibs of nondiabetic controls. This situation is not peculiar to diabetes for it is found in many other common diseases—duodenal ulcer, hypertension, rheumatoid arthritis, disseminated sclerosis and pernicious anemia, to mention but some of them—and it seems that once a disease becomes common its method of inheritance ceases to obey the simple Mendelian laws. Thus there are never nearly as many affected sibs as there ought to be, that is to say, as there would be if we were measuring the height of garden peas. To get over this difficulty the geneticist usually falls back either on the idea of incomplete penetrance or on that of multifactorial inheritance. By incomplete penetrance is simply meant that some individuals genetically liable to a disease do not, in fact, develop it. Now while it is easy to pour scorn on this "way out," yet there is nothing inherently improbable in the idea, and it can be shown in fact to be true in animal work where sometimes other genes or an environmental factor are necessary before the effects of a major gene can become manifest. In diabetes it would be reasonable to suggest that age, weight, parity and, to a slight extent, the ABO blood groups, might all influence the degree of penetrance of a gene or genes responsible for diabetes mellitus. By multifactorial inheritance we mean that a character is controlled by many genes, and when applied to diabetes it would mean that there is a continuous range from normal right through to the severest type of diabetes, the last having the complete "set" of genes influencing the character.

Now while every known type of inheritance has been invoked in diabetes, two principal views have been advanced to explain the majority of cases. Most favored is the recessive-gene-with-incomplete-penetrance hypothesis, but close behind it is the theory that the disorder is multifactorially controlled.

## THE RECESSIVE HYPOTHESIS

An affected individual is designated "dd" and if both his parents are unaffected they must both be of genotype "Dd." That is to say the mating will have been between two heterozygotes and if the penetrance were complete 25 per cent of the offspring would be affected. If one parent of an affected individual were a diabetic the mating must have been between dd × Dd, and in these circumstances half the offspring would be diabetic. Lastly, if both parents were affected all the offspring would eventually be expected to develop the disease. The ratios of affected offspring in these three types of mating are therefore 1:2:4.

Pincus and White[1] collected the sibs of 106 propositi where none, one or both parents were diabetic. They found abnormal glucose tolerance tests in 6.8 per cent, 17.9 per cent and 25 per cent respectively. This is a close fit (1:2.6:3.7) to the ratios expected on a single

Paper read in Glasgow at a Colloquium on Prediabetes, organized by the Medical and Scientific Section of the British Diabetic Association, October 1960.

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gene hypothesis, always assuming the individuals with abnormal glucose tolerance tests are in fact prediabetic.

Steinberg and Wilder,[8] in a similar survey, analyzed 1,981 consecutive patients and found 4.7 per cent, 11.4 per cent and 16 per cent of their sibs were diabetic (a ratio of 1:2.4:3.4) according to whether none, one or both their parents respectively were affected. These results are again in keeping with the single recessive gene hypothesis, the penetrance being much reduced—to about 25 per cent in Pincus and White's series and to about 18 per cent in Steinberg and Wilder's if the propositi are excluded.

On the other hand, Walker[5] in a complete survey of the Leicestershire village of Ibstock (population 5,500), found that 167 individuals (excluding the known diabetics) had postprandial glycosuria when given 50 gm. of glucose. All but 25 of these submitted to a glucose tolerance test and the curves obtained showed a gradual change from seventy-five normal individuals (who presumably had renal glycosuria) through an intermediate group of forty-two cases to twenty-five undoubted new diabetics.

Again, Keen, quoted by Pyke,[7] found that 25 per cent of the first degree relatives of diabetics had glycosuria one hour after consuming 50 gm. of glucose, but only 15 per cent of control relatives did so. The distribution of blood sugar levels in the relatives in the two groups showed no natural division between values regarded as normal and abnormal.

Steinberg (personal communication) does not think that the above findings invalidate the single-gene hypothesis. He points out that in fibrocystic disease of the pancreas (known to be controlled by an autosomal recessive gene) there is a wide range in the concentration of electrolytes in the sweat of the homozygotes, heterozygotes and normals. In a personal survey carried out with Shwachman he did not find evidence of bimodality when comparing the heterozygotes with normals, though the former have on the average twice the concentration of electrolytes compared with presumed normals. He thinks that a similar situation may occur with respect to the blood sugar in diabetes mellitus.

The analogy is of interest, but as far as fibrocystic disease is concerned his findings must surely mean that this particular character is controlled by many genes, one of them being the major gene for the disease, here acting as a polygene; the environment is also almost certainly of importance when considering a character such as the composition of the sweat. Steinberg contests this interpretation; he points out (personal communication, 1960) that the variation in eye size of the "eyeless" flies in *inbred* lines of Drosophila varies from 0 to 400 or more facets, and yet the character is controlled by a single gene. It seems to the writer that "eyeless" in Drosophila corresponds to dd in the diabetics, and that in diabetes the expression of the homozygous state is (as in the flies) very variable, from complete impenetrance to an acute fulminating disease. If Steinberg were to take families of Drosophila which segregated for "eyed" and "eyeless," he would invariably find a bimodal distribution for this character.

What is not certain is how close a relationship exists between the primary action of the gene or genes and the blood sugar levels. However, blood sugar curves are essential to the diagnosis of the disease, and all genetic hypotheses, including Steinberg's, have been related to them.

A priori, it seems probable that the blood glucose level is nearer to the primary action of the gene in diabetes than is the composition of the sweat in fibrocystic disease. In support of this it would be easy to pick out nearly all overt diabetics from nondiabetics by looking at the results of glucose tolerance tests only, whereas it would not be nearly so easy to distinguish heterozygotes from patients with established fibrocystic disease simply by a knowledge of the electrolyte content of their sweat.

Since these two lines of research seem to indicate different types of inheritance, it would be worth while carrying out a further and more extensive investigation. Thus a large number of students who have no family history of diabetes should be tested for the range of blood sugar values after a glucose tolerance test. Thereafter a series of young diabetics should be found since these will have sibs of approximately the same age as the students. Glucose tolerance tests would be carried out on these sibs and if the blood sugar is directly related to a single gene controlling diabetes one would expect bimodality. Thus those who were not genetically liable (both homozygote normals and heterozygotes) might give figures approximating to the controls, whereas some of the sibs who were of the genetic constitution dd would be "uncovered" by the test and would show an abnormal curve. The main point of the investigation is that by using sibs genetic variability and differences in environment are minimized; if the latter could be entirely removed the glucose tolerance test would be bound to show bimodality if a single gene is responsible for the disease. In our investigation it would also be of interest to find the ABO blood group of the sibs to see if those who were group A were more readily "uncovered" than those who were group

O. There is strong but by no means overwhelming evidence that diabetes mellitus is commoner in those belonging to group A than in those who are not A. The association is less strong than with pernicious anemia (see Fraser Roberts[6]).

If bimodality of blood sugar values were found it would be support for the single gene hypothesis. If, on the other hand, the range from normal to abnormal showed a continuous distribution it would suggest either that the single-gene hypothesis was wrong in that the condition was multifactorially controlled or that the blood sugar level was not the best index of the disorder, and that it was worth looking elsewhere for other criteria.

Whichever type of inheritance is correct, genetics cannot at present give accurate information regarding the liability of any particular individual to develop the disease since there is no reliable test which will invariably "uncover" the prediabetic state. All that we are really in a position to say at present is that the disorder runs in families, and that the more relatives that are affected the greater the chance of an individual to develop the disease.

SUMMARY

The two most widely accepted views on the inheritance of diabetes mellitus are discussed. Both the recessive gene hypothesis and the theory of multifactorial control appear to have some supporting evidence, but in neither case is this conclusive. An outline of further work which is being started in Liverpool may be of help in deciding between the two standpoints.

SUMMARIO IN INTERLINGUA

*Le Genetica de Diabete Mellite*

Es discutite le duo conceptiones del hereditate de diabete mellite que es le plus extensement acceptate. Tanto le hypothese de un gen recessive como etiam le theoria de multiple factores determinatori pare haber un certe supporto objective, sed isto es conclusive ni in le un ni in le altere caso. Un delineation de studios additional currentemente initiate in Liverpool forsan va adjutar in decider in favor del un o del altere del duo punctos de vista.

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## *Lipid Mobilizing Hormone*

The intricate mechanisms whereby the body regulates lipid metabolism continue to puzzle the imagination. The following is a unique concept.

In a summary of the past six years of studies, C. J. D. Zarafonetis, J. Seifter, D. H. Baeder and J. P. Kalas (*A.M.A. Arch. Int. Med. 104*:974, 1960) describe a factor which releases fats into the circulating blood. They found that hyaluronidase and partially depolymerized hyaluronic acid induce clearing of lipemic serum of animals and that cortisone inhibits this effect. Studies of the nephrotic syndrome disclosed the presence of a substance which the authors called "lipid mobilizer hormone" (LMH), which was prepared from the serum of horses previously treated with cortisone. This substance, which was dialyzable, and seemed to be an octapeptide, was found to release triglycerides from omental and mesenteric fatty stores (Seifter and Bader, *Proc. Soc. Exp. Biol. Med. 86*:709, 1954; *91*:42, 1956; 95:318, 469, 1957).

Single injections of LMH into fasting patients induced increases in concentrations of cholesterol, fatty acids and lipid phosphorus of the peripheral blood. Patients fed a diet low in fat and given daily injections of LMH for two weeks developed marked degrees of hyperlipemia (Zarafonetis et al. *Am. J. Med. Sci. 234*: 493, 1957). Prefeeding patients with glucose or fats prevented this hyperlipemic response, but prefeeding with amino acids did not. In animals, depletion of hepatic glycogen or exposure to substances injurious to the liver predisposed them to an exaggerated response to LMH. Evidence of endogenous secretion of LMH was obtained by studying patients during and after operation and animals subjected to various forms of stress. In these instances, a great increase in the concentration of venous fatty acids occurred, but the arterial concentrations did not increase. Cholesterol was affected to a lesser extent.

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# Survival of Diabetics with Proteinuria

*F. I. Caird, D.M., M.R.C.P., Birmingham, England*

The prognosis of diabetics with proteinuria is widely held to be very poor. This belief derives largely from postmortem studies, which must underestimate survival unless all cases seen in life are followed till death.[1] Nor is it remembered that the majority of patients with diabetic glomerulosclerosis are elderly,[2–4] and would be likely for that reason alone to have a short expectation of life. Studies of younger patients have in fact shown a substantially better prognosis, with an average survival in fatal cases of up to eleven years from the onset of proteinuria,[5–7] and an occasional patient living as long as seventeen years.[8]

The present study is an attempt to give a more accurate estimate of the survival of diabetics with proteinuria.

## MATERIAL AND METHODS

The records of all patients with proved diabetes who first attended the Diabetic Clinic at the General Hospital, Birmingham, between January 1946 and March 1949, were scrutinized. Irrespective of the presence or absence of other complications of diabetes all patients who at any time showed persistent proteinuria, of which the date of onset was known, were selected for follow-up. Proteinuria was defined as persistent if it was present in trace or greater amounts, as measured at first by boiling and more recently by salicylsulphonic acid, on two or more occasions separated by not more than three years. Its date of onset was considered known if urine tests had been performed at the clinic in the year before the first positive test and had shown no protein. Some thirty cases were excluded because the date of onset could not be defined. Two thirds of these had proteinuria at their first attendance at the clinic. The remainder had been absent from the clinic for over a year, and had proteinuria for the first time on their return. Since their return was often due to symptoms referable to renal disease, such as edema or uremic vomiting, their inclusion would introduce bias in favor of more severe cases.

Table 1 shows the sex distribution of the 134 cases with proteinuria as defined, and their ages both at the diagnosis of diabetes and at the onset of proteinuria.

The 134 patients were followed between March and October 1959, by reference to the hospital's records and by communication with general practitioners and with the General Register Office. All that was determined was whether each patient was alive or dead and, if dead, the date of death. Thirteen cases lost to follow-up were included up to the last date on which they were known to be alive. Survival was computed in complete months survived from the date of onset of proteinuria. A survival curve was constructed by the actuarial method.[9]

The natural, expected mortality[10] of a group of similar age and sex distribution to the patients at the time of onset of proteinuria was calculated from figures for the West Midlands conurbation for 1951.[11] Allowance was made for the effect of aging on mortality by reclassification of the patients into quinquennial age groups at the beginning of the second and each subsequent year, and separate calculation of the natural expected mortality for each year.[10]

An attempt was made to construct a control group of diabetics without proteinuria, matched for age, sex, and duration of diabetes. The limiting factor in the matching for duration of diabetes was the end-point of the period of follow-up. This end-point was related either to failure to continue attendance at the clinic, which is often associated with early subsequent death, or to attendance up to the present time, which is closely associated with survival. Controls matched by this method would thus show bias in one direction or the other with regard to survival and could not be used for comparison. There are no exactly comparable figures in the literature for the mortality rates of diabetics, and those of table 40 of Joslin et al.[12] for experience years 1947 to 1951 have therefore been used as the most suitable. Mortality rates for diabetics over seventy-five years of age were obtained by extrapolation on the assumption that the relation between mortality and age is exponential.

The rates used are given in table 2. A curve for the expected survival of a group of diabetics of age and sex distribution similar to the patients was constructed by a method identical with that described above, with an identical correction for aging.

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TABLE 1

Distribution by age and sex of 134 diabetics with proteinuria

| Age at | | Under 10 | 10-9 | 20-9 | 30-9 | 40-9 | 50-9 | 60-9 | 70-9 | 80+ | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Diagnosis of diabetes | M | 2 | 4 | 3 | 5 | 8 | 15 | 9 | 2 | 0 | 48 |
| | F | 0 | 2 | 1 | 0 | 13 | 27 | 34 | 9 | 0 | 86 |
| Onset of proteinuria | M | 0 | 1 | 4 | 2 | 9 | 8 | 18 | 6 | 0 | 48 |
| | F | 0 | 2 | 0 | 1 | 0 | 17 | 39 | 26 | 1 | 86 |

TABLE 2

Annual mortality rates of diabetics

| | Age: | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | 75 | 80 | 85 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mortality per 1,000 per year | M | 2.6 | 4.6 | 8.0 | 13.1 | 15.2 | 12.5 | 12.1 | 17.5 | 24.1 | 35.0 | 55.8 | 73.4 | 92.7 | 144 | 205 | — |
| | F | 2.6 | 4.6 | 8.0 | 13.1 | 15.2 | 12.6 | 12.3 | 14.6 | 19.0 | 29.4 | 43.7 | 56.5 | 79.8 | 117 | 167 | 238 |

Figures for ages under seventy-five taken from table 40 of Joslin et al.,[12] experience years 1947-51.
Figures for age seventy-five and over derived by extrapolation, on the assumption that the relation between mortality and age is exponential.

TABLE 3

Survival curves

| Years | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Survivors per cent: | | | | | | | | | | | |
| Natural expected | 100.0 | 97.0 | 93.9 | 90.5 | 86.8 | 83.0 | 78.9 | 74.7 | 70.3 | 66.0 | 61.5 |
| Diabetic expected | 100.0 | 94.6 | 89.2 | 83.7 | 78.3 | 72.8 | 67.2 | 61.8 | 56.4 | 51.2 | 46.0 |
| Diabetics with proteinuria | 100.0 | 93.9 | 87.6 | 79.1 | 70.3 | 64.6 | 52.1 | 39.5 | 31.0 | 27.9 | 27.9 |
| Standard error in per cent | — | 2.1 | 2.8 | 3.6 | 4.3 | 4.8 | 5.4 | 5.6 | 5.8 | 6.0 | 6.0 |

## RESULTS

Table 3 and figure 1 give the survival curve of the 134 patients with proteinuria, together with the standard error of each point on the curve, and the natural and diabetic expected survival curves. The survival rate of the patients with proteinuria is 65 per cent at five years and 28 per cent at ten. The corresponding natural expected survival rates are 83 per cent and 61 per cent, and the diabetic expected rates 73 per cent and 46 per cent. The number of patients in the younger age groups is too small, as table 1 shows, for calculation of a separate survival curve without an unacceptably large standard error to each point. But of the nineteen patients under the age of fifty at the onset of proteinuria, eight are known to have survived five years, and one nine years, while only four are known to have died.

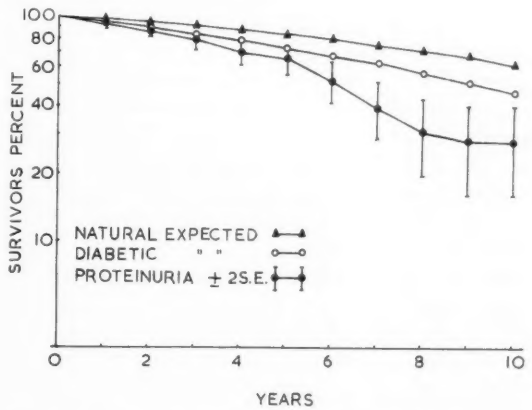


FIG. 1. Natural and diabetic expected survival curves, and survival curve of patients with proteinuria. Vertical bars give two standard errors above and before each point.

## DISCUSSION

The group of patients studied here is comparable to that described by O'Sullivan et al.[4] from the same clinic. They found the same relation between the age at diagnosis of diabetes and the duration of diabetes at the onset of proteinuria. But since the duration of diabetes was in the great majority of cases in the present series necessarily less than the thirteen years (1946 to 1959) covered by the study, there will have been a relative increase in the preponderance of older patients with proteinuria in the present group, since a higher proportion of older than younger patients who develop proteinuria do so within thirteen years of the diagnosis of diabetes.[4] With this exception the group studied here is thought to be representative of all diabetics with proteinuria.

Study of the case records alone did not allow any attempt to decide the cause of the proteinuria. In a small number of cases it was certainly not due to diabetic nephropathy, but in the great majority there is no reason to doubt that this was in fact the cause, as O'Sullivan et al.[4] find. Some support is lent to this view by the regularity of form of the survival curve, which suggests that it represents the survival of only one population. It may be concluded that either one etiologic group greatly predominates or that, if there is more than one etiologic group, all such groups have approximately the same prognosis.

The definition employed of the onset of proteinuria must result in inaccuracy in some cases, especially since proteinuria is often intermittent at first.[7] But since the onset of proteinuria will always tend to be postdated, the resulting error will be towards an underestimate of the duration of survival after the onset. Computation of survival from the date of onset will overestimate survival in the early months, since by definition no deaths can occur in the interval between the onset and the date of determination of the persistence of proteinuria. But since this interval was only a few months in the great majority of cases, the error introduced will be small. The survival rate obtained should thus be not far from the truth. The standard errors are not unduly large. The 95 per cent confidence limits, given by two standard errors above and below each point, are 55 per cent and 74 per cent for the five-year survival rate, and 16 per cent and 40 per cent for the ten-year rate. These findings show that the prognosis for life for diabetics with proteinuria is substantially better than has been believed in the past. This result is not unexpected, since long-term studies of living patients almost invariably give much better survival rates than investigations based on fatal cases alone.[10]

The method of construction of the natural, expected survival curve is such that it represents a reasonably close approximation to the truth. There is no such certainty about the diabetic, expected curve. Diabetics attending the Joslin Clinic undoubtedly differ from those attending the General Hospital Clinic in social and economic status, and thus very probably in the control of their diabetes. This must affect prognosis, but the magnitude of the difference is quite unknown. Nor can there be great confidence in the extrapolation necessary to provide mortality rates for diabetics over seventy-five years old. A further source of error is that the figures given by Joslin et al.[12] do not take account of the increase in mortality rates among diabetics that occurs with increasing duration of diabetes, especially perhaps after fifteen or twenty years, independently of and in addition to the increase expected because of aging alone. However, in the absence of more strictly comparable figures, those given must be accepted, with the reservations stated, as representing an approximation to the expected mortality of diabetics of an age and sex distribution similar to that of the patients with proteinuria.

Inspection of the survival curves shows that a large part of the mortality of diabetics with proteinuria is attributable to the effects of age alone, and to the increased mortality of diabetics compared with the general population. If the survival rates of the patients with proteinuria are expressed as percentages of the natural and diabetic expected survival rates,[1,13] the so-called "corrected" rates for five and ten years are 77 per cent and 44 per cent respectively of the natural, expected rates, and 89 per cent and 59 per cent of the diabetic's expected rates. These figures are further evidence that the prognosis of diabetics with proteinuria is better than is usually believed.

## SUMMARY

One hundred and thirty-four diabetics were followed for up to ten years from the onset of proteinuria. Their survival was compared with estimates of that expected of the general population and of all diabetics.

Sixty-five per cent survived five years and 28 per cent ten years from the onset of proteinuria. This represents 77 per cent of the natural expected rate for five years, and 44 per cent for ten years, and 89 per cent and 59 per cent respectively of the diabetic expected rate.

It is concluded that the prognosis for life of diabetics with proteinuria is substantially better than has been believed in the past.

SUMMARIO IN INTERLINGUA

*Le Superviventia de Diabeticos con Proteinuria*

Cento trenta-quatro diabeticos esseva tenite sub observation durante periodos de usque a dece annos post le declaration de proteinuria. Lor superviventia esseva comparate con illo a expectar in le population general e in le population de omne diabeticos.

Sexanta-cinque pro cento superviveva cinque annos e 28 pro cento dece annos a partir del declaration de proteinuria. Isto representa 77 pro cento del expectate cifra natural pro cinque annos e 44 pro cento del expectate cifra natural pro dece annos. In comparation con le population diabetic total, le correspondente proportiones es 89 e 59 pro cento, respectivemente.

Es concludite que le prognose pro le superviventia de diabeticos con proteinuria es substantialmente melior que lo que esseva credite in le passato.

ACKNOWLEDGMENT

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## *Effect of Chronic Hypothalamic Stimulation upon Cholesterol-induced Atherosclerosis in the Rabbit*

Preceding data appear to indicate that rabbits fed a diet excessive in cholesterol and cottonseed oil and, in addition, receiving chronic stimulation of their diencephalon in the vicinity of the ventral medial nucleus, the lateral and the anterior hypothalamic areas, will exhibit a greater degree of chronic lipemia and a greater degree of aortic and coronary atherosclerosis than their non-stimulated controls. . . .

The mechanism(s) whereby this chronic stimulation may have brought about the observed changes in the arterial vessel walls remains to be elucidated. However, it seems clear from the above study that they were not mediated by changes in food intake, by *chronic* changes in blood pressure or in physical activity. Our failure to observe any change in the corticosteroid level of the serum either during the period of chronic stimulation or soon (one to five hours) after acute stimulation suggests but does not prove that the adrenal corticosteroids did not play a very important role in the pathogenesis of the atherosclerosis found in these animals.

Perhaps the chronically sustained greater degree of lipemia observed in the stimulated animals was responsible for the more extensive aortic and coronary atherosclerosis found. Certainly this lipemia must have a significant role if only because identical hypothalamic stimulation of rabbits given ordinary rabbit laboratory chow failed to elicit any detectable change either in the blood lipids or in the arterial walls. The means whereby chronic diencephalic stimulation (under the conditions of the present study) might intensify the hyperlipemic response of rabbits fed controlled rations of lipid-enriched food also remains to be understood.

By C. G. Gunn, Meyer Friedman and Sanford O. Byers, in *The Journal of Clinical Investigation*, Volume 39, pp. 1963-72, Dec. 1960.

# Steroid Diabetes in the Cat

## Protection of the Islets by Insulin or Phlorhizin

*F. D. W. Lukens, M.D., Stanley N. Cohen, M.D., and Yoshio Goto, M.D., Philadelphia*

In a preliminary report, Buse, Gundersen and Lukens[1] indicated the frequency with which 9α-fluorohydrocortisone (FLF) caused glycosuria in normal cats. In addition, two normal cats given prolonged and repeated courses of steroid treatment showed some degree of atrophy of the islands of Langerhans resembling that seen in metahypophyseal diabetes in this species. The general effects of this drastic steroid treatment were described as well as the effects of steroid diabetes on the islands.

During its administration FLF causes marked adrenal cortical atrophy which persists for weeks or months after the termination of treatment, so that considerable islet damage might be present without diabetes in the metasteroid period. Because of this, studies requiring less prolonged treatment have been undertaken to ascertain the behavior of the islands in steroid diabetes. Furthermore, partial pancreatectomy without spontaneous diabetes has been used to prepare animals which could be made diabetic more consistently and with smaller doses of steroid. The results, which add another means of producing diabetes with concomitant injury of the islets of Langerhans in this species, will be compared with earlier studies of pituitary diabetes.[2,3]

### METHODS

The operative and chemical methods as well as the conduct of the metabolic studies have been described.[1,2] In some of the present experiments blood glucose has been determined by the glucose oxidase[4] instead of the Nelson method.[5] In all metabolic periods the daily diet of canned horse meat (Cadillac Dog Food, B. A. Bernard & Company, Philadelphia) was weighed and given throughout the periods recorded. Analysis of the meat for nitrogen and the use of the conventional conversion factors (N × 6.25 × 0.58) gave a value for available carbohydrate of the order of 10 gm. per 100 gm. meat. The percentage of the available glucose of the diet which was excreted in the urine has been used in table 2 as an index of severity of the diabetes.[6,7]

We are grateful to E. R. Squibb & Sons and Merck, Sharp and Dohme, who provided the 9α-fluorohydrocortisone as a microcrystalline suspension, as well as other steroids which were briefly tested. Commercial insulin (Lilly) and phlorhizin (Phloridizin) prepared by the Nutritional Biochemicals Corporation were used in the studies on treatment. Phlorhizin was administered subcutaneously in suspension in warm olive oil. Because of the prolonged absorption of the oil suspension, the usual dose of 0.5 gm. was effective when administered every second or third day.

### RESULTS

*Steroids tested.* 9α-fluorohydrocortisone (FLF) has been the most consistent and potent diabetogenic agent in the cat. Cortisone (50 mg./day); hydrocortisone (5 to 10 mg./day); prednisolone (5 mg./day) and $\Delta^1$-9α-fluorohydrocortisone all failed to produce glycosuria in partially depancreatized cats which at other times readily became diabetic when given FLF. Prednisone (50 mg./day) caused marked glycosuria in one animal tested.

*The relation of degree and duration of hyperglycemia to the development of island lesions* is shown in table 1. Twenty-one courses of FLF in eighteen animals have been arranged in three broad groups according to the level of blood glucose and the appearance of the islands at the end of the treatment periods. In table 1, the duration of hyperglycemia refers to the period from the first elevated level until the biopsy. The true period of hyperglycemia was probably a few days longer than this because blood sugars were not determined until glycosuria had been present for a few days. In contrast, the transitory hyperglycemia of cats 638, 688, and 698 is indicated by the final blood sugar values and the duration of hyperglycemia was therefore less than the nineteen, twenty and twenty-four days (respectively) from the first elevated blood sugars. When this is taken into account, table 1 shows that the administration of steroid

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TABLE 1

Relation of duration and degree of hyperglycemia to islet lesions in cats treated with fluorohydrocortisone (FLF)

| Cat no. | FLF mg. times days | Blood glucose: Elevated days | No. | Range mg. per 100 ml. | Final | Islands |
|---|---|---|---|---|---|---|
| 568 | 2.5×18 | 1 | 1 | | 122 | Normal |
| 588 | 2.5×24 | 1 | 1 | | 188 | " |
| 628b* | 2.5×27 | | 1 | | 80 | " |
| 638 | 2.5×31 | 19 | 5 | 230-128 | 128 | " |
| 648 | 2.5×30 | 1 | 1 | | 138 | " |
| 688 | 2.5×30 | 20 | 5 | 194-73 | 73 | " |
| 548 | 2.5×28 | 1 | 1 | | 168 | Degran. |
| 698 | 2.5×30 | 24 | 6 | 237-106 | 106 | " |
| 384a† | 5×27 | 23 | 7 | 100-194 | 194 | " |
| 634 | 10×40 | 23 | 4 | 130-191 | 191 | " |
| 247 | 5×29 | 18 | 5 | 150-195 | 152 | " |
| 384b† | 2.5×89* | 85 | 18 | 100-280 | 160 | Hydropic |
| 307 | 2.5×33‡ | 44‡ | 6 | 130-300 | 300 | " |
| 337 | 1.2×38 | 28 | 8 | 132-237 | 217 | " |
| 367 | 1.2×23 | 19 | 6 | 223-370 | 370 | " |
| 377 | 2.5×29 | 22 | 7 | 162-284 | 240 | " |
| 447 | 2.5×29 | 12 | 4 | 200-233 | 200 | " |
| 457 | 2.5×35 | 24 | 3 | 200-204 | 200 | Degran. |
| 538 | 2.5×40 | 34 | 4 | 224-370 | 370 | Hydropic |
| 628a† | 2.5×34 | 22 | 6 | 184-299 | 232 | " |
| 678 | 2.5×39 | 28 | 9 | 121-193 | 161 | " |

All animals had partial pancreatectomy and had recovered without diabetes before experiments were begun.

*628a—Biopsy performed after thirty-four days of first period of steroid administration.

628b—After a sixteen-day recovery period, a second course of steroid failed to cause glycosuria.

384a—Biopsy at first twenty-seven days of eighty-nine-day course of FLF. Second biopsy on ninetieth day and all blood sugars of the eighty-nine-day period are recorded under 384b.

‡Biopsy performed eighteen days after the last FLF but with continued hyperglycemia.

without sustained hyperglycemia caused no visible alteration in the islands. The second category, degranulation, was associated with sustained hyperglycemia of two to three weeks' duration in which several values fell below 200 mg./100 ml. The third group, hydropic change (glycogenization) of the β cells, was in general associated with the combination of long duration and higher degree of hyperglycemia. Some figures below 200 mg./100 ml. occurred and in the case of cat 457 only three determinations were made and lower levels of blood glucose may have occurred at times. It would be a mistake to associate level of blood glucose, duration of hyperglycemia and island lesions in any rigid fashion. Nevertheless, the correlation between degree and duration of hyperglycemia and damage of the islands is clear in spite of these irregularities. In relation to the duration and sustained level of hyperglycemia, the breakdown of the β cells in these experiments, in which a steroid was the diabetogenic agent, follows the pattern observed in pituitary diabetes in this species.[2,3]

*Effect on the islands of altering the level of blood glucose by treatment.* Table 2 shows the results of treatment with insulin or phlorhizin. In the case of the cats treated with insulin, FLF only was first administered for the periods stated, after which pancreatic biopsy was performed. After this, insulin treatment was begun and insulin and FLF were continued until the second examination of the pancreas at autopsy (see figure 1). The effect of insulin is indicated by the reduced duration and degree of glycosuria and by the selected blood glucose values. In each animal, islands damaged during steroid diabetes were restored by insulin treatment. In the case of 337 and 538, an occasional hydropic cell remained but the essentially complete recovery has been tabulated as normal. Figure 1 illustrates one of these experiments, in which a partially depancreatized cat was treated with FLF. In a few days hyperglycemia and glycosuria developed, and diabetes was sustained until the biopsy of the pancreas on the thirty-ninth day. After the biopsy, steroid was continued in the same dosage but insulin treatment was begun with the reduction in glycosuria and hyperglycemia which is apparent. The final high blood sugar was attributed to some error in the previous dose of insulin and does not alter the fact that a lowering of blood sugar was the feature of this period of treatment. With this course of events in mind, the appearance of the islands before and after insulin treatment is presented in figures 2 and 3. At biopsy (figure 2) the islands showed marked hydropic degeneration of the β cells. At autopsy after insulin treatment (figure 3) anatomical recovery is apparent. As figure 1 shows, this restoration occurred during the continued administration of the diabetogenic agent, a result which means that the islet injury is not a direct toxic effect of the steroid, but is a consequence of the diabetic state.

Figure 4 illustrates an experiment done in the reverse order in which phlorhizin was the therapeutic agent. After partial pancreatectomy without diabetes a few days of steroid treatment caused glycosuria and a single elevated blood sugar was recorded to complete the demonstration that this animal responded to FLF with diabetes. Treatment was then begun with phlorhizin, which caused a striking lowering of the blood sugar and the expected glycosuria by its action on the renal tubules. After the biopsy there was a rest period of twenty-seven days during which the blood sugar was normal and glycosuria ceased. When treatment was resumed with FLF alone, diabetes developed and continued until autopsy. At biopsy the islands were normal in appearance (figure 5) and at autopsy they showed distinct hydropic change (figure 6). As in the case of insulin, the protection from

TABLE 2

Restoration or prevention of island lesions by treatment with insulin or phlorhizin

| Cat no. | Treated or untreated | Duration of FLF | Duration of glycosuria | Dietary glucose excreted* | Blood glucose (last two values of period) | Island histology (end of period) |
|---|---|---|---|---|---|---|
| | | Days | Days | Per cent | Mg./100 ml. | |
| | *Steroid diabetes* | | | | | |
| 337 | Before insulin | 38 | 34 | 74 | 217, 211 | Hydropic |
| | During insulin | 27 | 17 | 8 | 56, 78 | Normal |
| 447 | Before insulin | 30 | 19 | 31 | 233, 200 | Degran. |
| | During insulin | 11 | 0 | 0 | 142, 53 | Normal |
| 538 | Before insulin | 40 | 37 | 17 | 280, 370 | Hydropic |
| | During insulin | 27 | 4 | 0 | 235, 12 | Normal |
| 377 | Before phlorhizin | 5 | 3 | 50 | —, 191 | — |
| | During phlorhizin | 18 | 18 | 100 | 117, 116 | Normal |
| | After phlorhizin | 29 | 24 | 38 | 256, 240 | Hydropic |
| 457 | Before phlorhizin | 36 | 32 | 14 | 202, 200 | Degran. |
| | During phlorhizin | 17 | 17 | 51 | —, 156 | Normal |
| | *Spontaneous diabetes* | | | | | |
| 367† | Control period | 0 | 8 | 42 | 205, 230 | — |
| | During phlorhizin | 0 | 15 | 74 | 53, 53 | Normal |
| | After phlorhizin | 23 | 22 | 64 | 275, 370 | Hydropic |

All animals had recovered from partial pancreatectomy and some were free of glycosuria before FLF treatment was begun, with the exception of 367 (control period). Diet of each animal was constant.

*The proportion of the available glucose of the diet which was excreted in the urine is recorded as an index of the severity of the diabetes. Figures are the averages of the last six to seven days before biopsy or autopsy.

†Cat 367, diabetic after partial pancreatectomy, recovered after phlorhizin treatment. FLF was begun after twenty-seven days without glycosuria or hyperglycemia.

island injury took place in the presence of the diabetogenic steroid.

A third experiment, performed in part by nature, may be mentioned. Cat 628a and b (see table 1) had a first course of FLF, became diabetic (628b), and at thirty days had hydropic degeneration as shown in figure 7. After a rest period during which the animal failed to regain its weight, a second course of the same duration and dosage of steroid was given. This time there was no diabetes (628a) and at autopsy the islands were normal (figure 8). Thus in the same cat, almost identical courses of steroid first did and later did not cause injury of the islets depending on whether or not diabetes was produced. One can only speculate as to why this animal, like a few others, failed to respond. The development of antibodies to steroids seems most unlikely, but cachexia in this animal may have led to a failure of "end organ responsiveness." Another possibility is that an islet cell adenoma may have developed, for this has been seen occasionally in the cat, but none was found at autopsy.

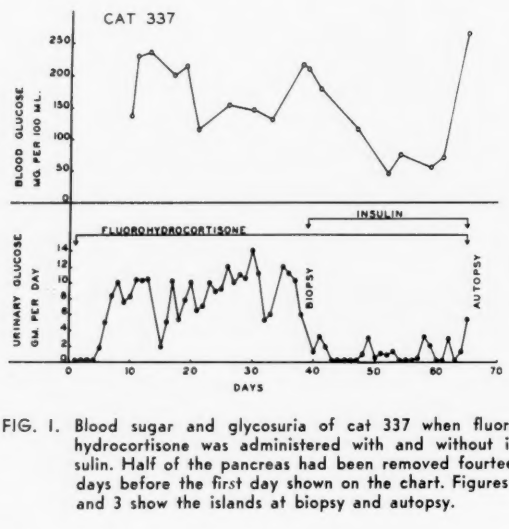


FIG. 1. Blood sugar and glycosuria of cat 337 when fluorohydrocortisone was administered with and without insulin. Half of the pancreas had been removed fourteen days before the first day shown on the chart. Figures 2 and 3 show the islands at biopsy and autopsy.

*Response of steroid diabetes to changes in diet.* These experiments were begun with the idea that steroid diabetes could be prevented or treated by suitable reductions in diet. Glycosuria has not been produced in the fasted animal, and it was assumed that marked changes in diet would influence the diabetes greatly. When put to the test, as seen in table 3, the glycosuria was never

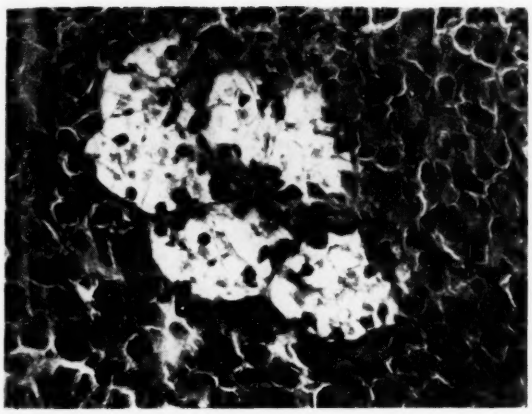

FIG. 2. Photomicrograph of hydropic degeneration of island of cat 337 from biopsy on day 39 (see figure 1) X 325.

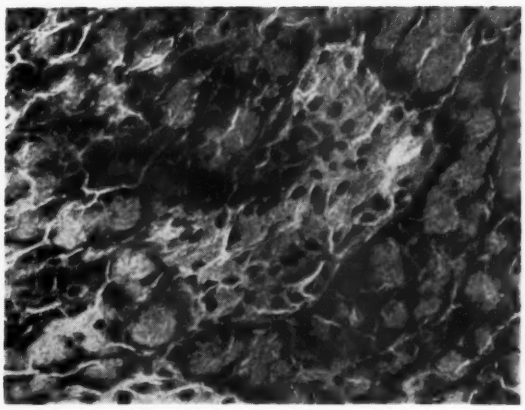

FIG. 3. Photomicrograph of island of cat 337 at autopsy (see figure 1) X 325.

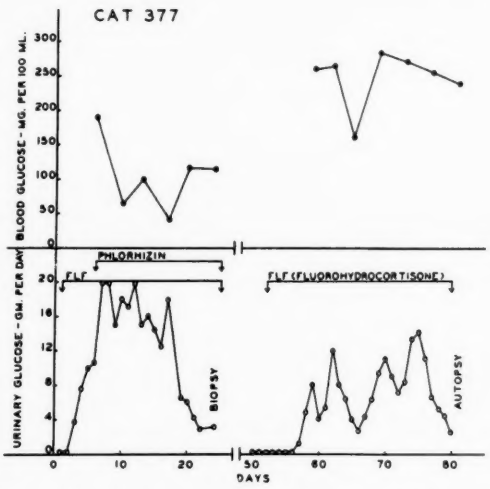


FIG. 4. Blood sugar and glucose excretion of cat 377 when fluorohydrocortisone was administered with and without phlorhizin. Partial pancreatectomy was performed nineteen days before the first day of this protocol. The break in the horizontal scale represents a rest period of twenty-seven days. Figures 5 and 6 show the islands at biopsy and autopsy.

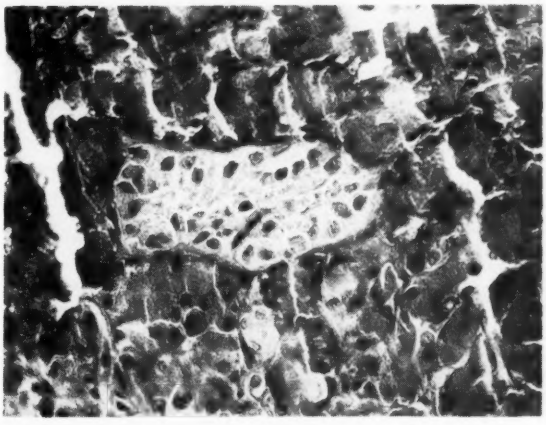

FIG. 5. Photomicrograph of island of cat 377 from biopsy on day 24 (see figure 4) X 350.

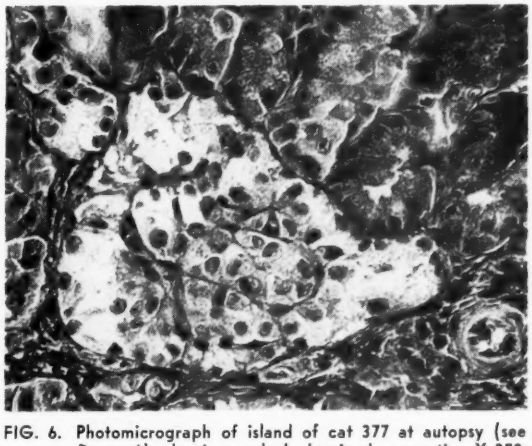

FIG. 6. Photomicrograph of island of cat 377 at autopsy (see figure 4), showing early hydropic degeneration X 350.

changed by the estimated 10 gm. of available glucose which was taken from or added to the diet. Even when the excretion of glucose was slight, as in most of these animals, there was no noteworthy change in the hyperglycemia on reducing the diet. These observations are not surprising when one reviews the previous studies of pituitary diabetes in the dog and cat.[2,6,7] For comparison, table 4 gives the response of a single diabetic patient to some changes in diet. The response of glycosuria to diminution of available dietary carbohydrate is seldom

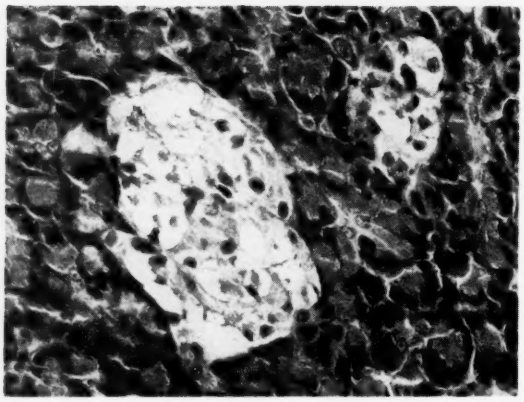

FIG. 7. Photomicrograph of hydropic degeneration from biopsy of cat 628 (see 628a, table 1) X 350.

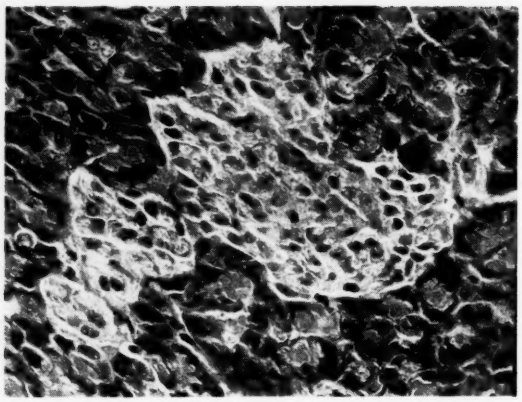

FIG. 8. Photomicrograph of island of cat 628 at autopsy (see 628b, table 1) X 350.

TABLE 3

Steroid diabetes: Response to changes in diet*

| Cat no. | FLF treatment Period† Days | Dose Mg./day | Diet (meat) Gm./day | Average glycosuria Gm./day | Blood glucose Day | Blood glucose Mg./100 ml. |
|---|---|---|---|---|---|---|
| 48 | 26-32 | 2.5 | 200 | 4.0 | 29 | 185 |
| | 33-39 | 2.5 | 100 | 3.0 | 40 | 205 |
| 78a | 12-18 | 2.5 | 200 | 6.7 | 17 | 167 |
| | 19-25 | 2.5 | 100 | 6.0 | 23 | 197 |
| 78b‡ | 16-22 | 1.2 | 150 | 9.7 | | |
| | 23-29 | 1.2 | 50 | 3.6 | | |
| 108 | 8-14 | 2.5 | 200 | 5.6 | 13 | 238 |
| | 15-21 | 2.5 | 100 | 1.8 | 19 | 179 |
| 148 | 13-19 | 2.5 | 200 | 4.8 | | |
| | 20-26 | 2.5 | 100 | 3.9 | | |
| | 27-33 | 2.5 | 200 | 9.2 | | |
| 168 | 18-24 | 1.2 | 200 | 13.2 | | |
| | 25-31 | 1.2 | 100 | 8.2 | | |
| | 32-37 | 1.2 | 200 | 12.6 | | |

*Each change in diet amounts to 100 gm. meat estimated to alter the available carbohydrate by 10 gm. Note that glycosuria never changes by this much.

†Both days inclusive indicating consecutive six- or seven-day metabolic periods within the course of steroid (FLF) treatment.

‡Second course of steroid treatment after a rest period without diabetes.

a mathematical relation. It appears to be influenced by several poorly measured factors, such as the degree of insulin deficiency, the activity of contra-insulin or diabetogenic agents and no doubt other things. The results in table 3 give the impression that even when the glycosuria is slight and the animal can recover promptly on stopping steroid, this diabetogenic agent creates a metabolic situation which is unexpectedly independent of or resistant to these changes in diet.

TABLE 4

Response to diet of a patient with mild diabetes from Graefe[8]

| Date (1929) | | Diet Total calories | Avail. COH Gm./day | Urinary glucose Gm./day | COH utilized Gm./day | Blood glucose Mg./100 ml. |
|---|---|---|---|---|---|---|
| April | 24 | 1510 | 97 | 37 | 60 | 187 |
| | 25 | 1517 | 67 | 27 | 40 | |
| | 26 | 1023 | 41 | 4 | 37 | 178 |
| | 27 | 1196 | 65 | 0 | 65 | |
| | 28 | 1521 | 139 | 6 | 133 | |
| | 29 | 1468 | 145 | 5 | 140 | |
| | 30 | 1800 | 138 | 10 | 128 | 99 |
| May | 1 | 1836 | 139 | 8 | 131 | |
| | 2 | 1050 | 43 | 0 | 43 | |
| | 3 | 1680 | 130 | 0 | 130 | |
| | 4 | 1850 | 142 | 0 | 142 | 105 |

## DISCUSSION

It remains to relate these findings to those obtained by other means and under other conditions. First, table 5 summarizes some of the facts about pituitary diabetes in the cat. Treatment of early diabetes, i.e., less than three months in duration, led to anatomical and functional recovery of the islands.[2,3] Disregarding the few experiments with adrenalectomy, reduction in diet, treatment with insulin or with phlorhizin were effective in reversing island damage. The results showed that diabetes, and not pituitary extract itself, was the damaging condition. Table 6 enlarges this picture by listing the four diabetogenic procedures which have been used in the cat, such as partial pancreatectomy, pituitary extract, massive injections of glucose and steroids. These four forms of experimental diabetes have all been prevented or alleviated by diminution in diet, treatment with insulin or treatment with phlorhizin. When it prevents

TABLE 5

Pituitary diabetes in the cat, treated in first three months

| Treatment | Results | | | |
|---|---|---|---|---|
| | Island structure | Island function | Blood sugar | Glucose retained |
| Low diet | Largely restored | Recovered | Became normal | Increased |
| Insulin | | Recovered | | |
| Adrenalectomy | | Recovered? | | |
| Phlorhizin | | Recovered | | |

TABLE 6

Prevention of experimental diabetes

| Means of prevention | Diabetogenic procedure |
|---|---|
| Low diet | |
| Insulin | |
| Phlorhizin | Partial pancreatectomy |
| Tolbutamide[8,9] | Ant. pituitary extract (APE) |
| | Glucose injections |
| Refractory to agent (APE) | Steroids |
| Unresponsive to agent | |
| (a) Cachexia? | |
| (b) Species | |

hyperglycemia, tolbutamide has also protected dogs from pituitary diabetes[9,10] but has not counteracted steroid diabetes in rabbits.[10] It is tabulated as another factor to be reckoned with in such experiments.

Other circumstances which may protect the islands from the action of diabetogenic agents are also suggested in the lower left compartment of table 6. Refractoriness to a protein such as growth hormone is usually attributed to antibody formation, which we have assumed does not apply to steroids while admitting the possibility of steroid:protein complexes. Unresponsiveness of an animal due to cachexia or other unknown reasons constitutes a large area of ignorance. Increased islet function has not been measured in the animals that tolerated steroid without diabetes in our experiments. The response, or lack of response, of different species to a given diabetogenic agent enables one to recognize two aspects of such agents, which may be called extrapancreatic and pancreatic or insular. The extrapancreatic differences in the reaction of different animals may be illustrated by the fact that pituitary extract or growth hormone is diabetogenic in the dog and cat but not in the rat as noted by Engel and his associates[11] and others. Likewise, cortisone diabetes, which was first described in rats by Ingle,[12] has been produced with difficulty, if at all, in dogs and cats as reviewed and studied by Abelove and Paschkis.[13] The effectiveness of fluorohydrocortisone in cats suggests that these differences may be due to the relatively low potency of the native steroids rather than to independence from steroid action. West[14] has reported marked differences in the diabetogenic effect of different steroids in man. The reasons for these differences in the systemic response of various animals are obscure but are distinct from the behavior of the islands themselves. Thus, there are differences in the sensitivity of the β cells to alloxan, which readily causes diabetes in rats, rabbits and dogs whereas the cat has not been made diabetic by alloxan in this laboratory. Other differences in islands of Langerhans under different circumstances will be noted below.

Lazarus and Volk[9] accept the fact that prolonged hyperglycemia is responsible for β-cell injury in cats and dogs and they note that two of their dogs which did not become diabetic on treatment with growth hormone suffered no noteworthy island lesions. We fully agree with their statement that "the mechanism whereby hyperglycemia causes β-cell destruction in the dog is, however, not known." They regard as unlikely "functional exhaustion," words which for years have emphasized the fact that chemical or biological definition of the demands placed on the β cells is lacking. However, the statement that functional demand usually leads to hyperplasia must be made with such points as the following in mind.

(a) Functional stress has been credited with the production of dietary nephritis in unilaterally nephrectomized rats by Medlar and Blatherwick[15] who wrote: "In the main the injury is sufficient to cause a considerable hyperplasia of the epithelium. This phenomenon is followed later by necrosis and dissolution of the hyperplastic tissue. The whole phenomenon may be regarded as an overwork of the epithelium." The production of the arteriolar lesions of experimental hypertensive retinopathy[16] now seems established as a response of the vessels to a form of functional demand. Whether such effects are encountered in islands or arterioles, exhaustion or overwork ought eventually to be defined in better terms.

(b) The dog and cat have a limited capacity for islet hyperplasia in comparison to the rat. However, the rat's ability to increase the size and insulin production of its islands is not seen under all circumstances. Whereas normal rats respond to glucose feedings with hypertrophy and hyperplasia,[17] rats made diabetic by partial pancreatectomy or alloxan commonly fail to overcome diabetes by island growth and this in the presence of enough insulin-secreting tissue to permit long survival and maintenance of weight. Whether or not the rat responds with hyperplasia, its β cells do not undergo hydropic degeneration (glycogenization) of the type and extent occurring in the dog and cat.

(c) Finally, if growth hormone and steroid diabetes produce island injury in the dog or cat by their hyperglycemic effect, one should consider the relations between the β-cell stimulating effect of glucose and that of the sulfonylureas. First, it is possible that glucose and sulfonylureas stimulate the β cells by different mechanisms. Secondly, whatever the mechanisms may be, the quantitative difference between glucose and the sulfonylureas in evoking insulin secretion is considerable. In man, the hypoglycemic curve of one dose of tolbutamide is matched by the response to 2 to 4 units of insulin.[18,19] With this there is practically no arteriovenous glucose difference in contrast to the large arteriovenous difference which follows a sugar tolerance test. The report of Yalow and Berson[20] on the marked difference in blood insulin levels after these two stimuli further defines the relatively strong effect of glucose and the limited potency of tolbutamide on the islands. Finally, the capacity of tolbutamide to sustain its stimulating effect (or of the β cell to maintain its responsiveness to the drug) is not yet understood.

After noting the differences in the functions of the islands in different species [see (b) above] one may ask, what is known of the behavior of the islands in man? The lesions at necropsy have been studied and the insulin content and changes in granulation have been described. There is little knowledge of what the islands can do in the earlier period of the diabetic's life. In an effort to observe this, Lukens and Dyer[21] reported the histologic appearance of the islands in three diabetic patients from whom adrenal tumors or hyperplasia were surgically removed. The first was a woman with pheochromocytoma and mild diabetes. At operation a medullary tumor was removed and a section of pancreas taken for biopsy. This showed slight but distinct hydropic degeneration of the islets. The operation was followed by immediate cessation of diabetes and a fully normal glucose tolerance test six months later. The second patient had mild diabetes controlled by diet alone. When a pheochromocytoma developed she required 55 units of insulin daily. At the time of excision of the medullary tumor, biopsy revealed islands which were described as "normal" and this patient continued to be frankly diabetic but again was controlled on diet only. The third patient had severe Cushing's syndrome and biopsy of the pancreas was performed when the second adrenal gland was removed. On maintenance adrenal cortical therapy her insulin requirement fell from 55 to 20 units daily. Microscopic examination showed a mixed picture of normal, hyperplastic and hyalinized islands in connection with this response of the diabetes. It is obvious that the islands of Langerhans in man display a variety of appearance and behavior which demands far more study than the chance findings in three patients.

## SUMMARY

Partially depancreatized cats without diabetes have been used to study steroid diabetes produced by the daily subcutaneous administration of 9α-fluorohydrocortisone. The general relations between duration and degree of hyperglycemia and the production of hydropic degeneration (glycogenization) of the β cells have been outlined. The prevention or restoration of such island damage by treatment with insulin or phlorhizin in the presence of continued administration of steroid has demonstrated again the injurious effect of diabetes (hyperglycemia) on the islands in this species. The results have been briefly related to those obtained in other forms of experimental diabetes.

## SUMMARIO IN INTERLINGUA

*Diabete per Steroides in le Catto: Protection del Insulas per Insulina o Phlorhizina*

Partialmente dispancreatisate cattos sin diabete esseva usate pro studiar diabete steroidigene producite per le diurne administration subcutanee de 9(alpha)-fluorohydrocortisona. Le relation general inter le duration e grado de hyperglycemia e le production de degeneration hydropic (glycogenisation) del cellulas beta es delineate. Le prevention o le restauration de tal injurias in le insulas per tractamento con insulina o phlorhizina in le presentia del continue administration de steroide ha demonstrate de novo le injuriose effecto de diabete (hyperglycemia) super le insulas in iste specie. Le resultatos es relationate in breve con le resultatos obtenite in altere formas de diabete experimental.

## ACKNOWLEDGMENT

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## *Effect of Carbohydrate Source on Cholesterol and Cardiovascular System*

Two years ago dietary lactose was found to increase the intestinal absorption of cholesterol in the rat (W. W. Wells, *Fed. Proc. 16*:402, 1957; Wells and S. B. Cooper, *Arch. Biochem. Biophys.* 75:273, 1958). This group (Wells and S. C. Anderson, *J. Nutrition 68*:541, 1959) has now taken a logical step forward in these investigations by contrasting the atherogenic effect of lactose with that of sucrose (29.35 per cent) in diets of rabbits supplemented with 0.35 per cent cholesterol (eighteen animals in each group). Pathologic lesions were correlated with levels of cholesterol in the animals' sera and livers. These various food mixtures all contained cottonseed oil (13 per cent) and cod liver oil (2 per cent) as the dietary fat. Soybean meal (45 per cent) was used in place of casein as the source of protein because it had been found previously that rabbits of both groups consumed the diet best when soybean was used. All animals gained weight under these conditions, those receiving the lactose-containing diet to a somewhat lesser degree than those in the sucrose-fed group.

Despite this lesser degree of weight gain by animals in the lactose group, significantly more severe degrees of atheroma were found on gross examination of their aortas seven to eight weeks after initiation of these dietary regimens than were present in animals in the sucrose group. Measurements of surface areas of intimal lesions by three independent observers revealed that changes in the vessels of the lactose-fed rabbits were five times as extensive as those in the aortas of rabbits fed sucrose. Cholesterol contents of livers and sera in the latter were, as might be expected, significantly lower than in the rabbits on the lactose-containing diets.

The authors point out that the increased atherogenesis in the lactose group could not be ascribed to a higher absolute intake of cholesterol because this group actually consumed 2 to 4 gm. less of the diets daily than did the rabbits in the sucrose group. Differences between the two groups in the amounts of cholesterol in sera and livers as well as in the relative severity of aortic atheromatous changes were far too great to be ascribed to either physiologic or chance variation.

Intestinal motility was shown, by a special technic, to be greater in the lactose-fed animals than in the sucrose controls, although diarrhea was absent in both groups. Therefore, the greater absorption of cholesterol from the intestines of the lactose-fed animals could not be attributed to lesser motility. The authors suggest that a contaminant of the lactose used (possibly lactaminic acid) might have increased cholesterol absorption, and they are repeating their study using a purified lactose. We hope it will be possible to measure cholesterol absorption more directly in these new experiments.

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# Serial Studies of Renal Function in Pregnancy Complicated by Diabetes Mellitus

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## INTRODUCTION

In normal pregnancy there occur marked endocrine and physiological readjustments which are associated with hypertrophy of renal function. Renal plasma flow is increased approximately 50 per cent and glomerular filtration rate roughly 30 per cent during midpregnancy.[1,2] The young or juvenile diabetic is subject not only to the effects of intercapillary glomerulosclerosis during pregnancy, but also to increased incidence of pyelonephritis and of toxemia of pregnancy.

It is known that diabetic patients may enter pregnancy with impaired renal function as a result of glomerulosclerosis and nevertheless successfully complete pregnancy without further deterioration of renal function, but the presence of such impairment is generally considered to place the outcome of the pregnancy in a poor prognostic category.[3] However, no data are available concerning the course of pregnancy with respect to renal function in such patients. In fact there is an element of uncertainty in many of the reported kidney function studies in diabetic subjects since precautions have not been taken to avoid interference of urinary glucose in the measurement of clearance of para-aminohippurate. The present renal function studies were carried out in order to answer in part the following questions: (1) Is the recent or the long-standing diabetic capable of a hypertrophy of renal function comparable to the normal? (2) When increase in filtration rate does occur in the diabetic, what effect does this have upon the renal threshold for glucose? (3) What pattern of renal function is found during pregnancy in those patients with intercapillary glomerulosclerosis, and what is the effect of pregnancy on the course of the disease?

## MATERIAL AND METHODS

*Experimental and Control Subjects.* The clinical findings in the group of eight patients studied are given in table 1. With three exceptions (cases 1, 3 and 8) these patients had shown gross evidence of vascular or renal complications of their diabetes. The previous control of the majority had been indifferent or poor even if judged by liberal standards. More than half had absent patellar reflexes, suggesting diabetic neuropathy. Control subjects were as previously described.[2,4]

*Procedure.* The technic of renal function studies was as previously described for studies of normal subjects in this laboratory,[2] with the exception that infusions of insulin and of para-aminohippurate (PAH) were freshly prepared in dextrose and water instead of saline. The patients were asked to take three quarters of their usual dose of insulin on the morning of the procedure and were given ginger ale during the procedure to insure some degree of hyperglycemia for the measurement of tubular reabsorption of glucose. An examination of the optic fundi and a culture, colony count and routine examination of urine were made at the time of each study. Except in early pregnancy, the patients were shifted into the lateral position during the clearance studies to minimize postural effects.

*Analytical Methods.* These were as previously described[2] except that the method of Bratton and Marshall was substituted for that of Brun in the determination of PAH.[5] It was found that PAH, when exposed to glucose in the urine of diabetic patients, undergoes Schiff-base conjugation, so that the apparent content of free PAH is reduced. For this reason, hydrolysis of any conjugate present was carried out by adding 1 ml. of 1.2 N. hydrochloric acid to each 5 ml. of filtrate or of diluted urine and by heating at 100° for seventy-five minutes. It had been shown that maximal hydrolysis of PAH which had been exposed to glucose was achieved within fifty minutes. Failure to take this precaution in patients with glucosuria may result in falsely low values for clearance of PAH. The method and calculations for determining renal tubular reabsorption of glucose and for correction of the inulin analyses for interference by glucose were the same as previously reported from this laboratory for the determination of $Tm_G$.[4] One milligram of glucose was found to be equivalent to 0.014 mg. of inulin.

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TABLE 1
Clinical findings in eight patients with pregnancy complicated by diabetes mellitus

| Patient | 1* L.B. | 2 S.K.B. | 3† L.H. | 4 C.H. | 5†‡ V.P. | 6 L.Sta. | 7† D.S. | 8 S.W. | Mean and range |
|---|---|---|---|---|---|---|---|---|---|
| Age | 23 | 22 | 26 | 22 | 23 | 30 | 21 | 26 | 24.1(21-30) |
| Duration of known diabetes —years | 10 | 17 | 8 | 11 | 17 | 13 | 13 | 23 | 14(8-23) |
| Degree of control | fair | good | poor | fair | poor | poor | poor | fair | |
| Ketoacidosis§ | | | | | | | | | |
| Previous | 0 | 0 | 2 | 0 | 1 | 3 | 3 | 1 | |
| Current | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | |
| Patellar reflexes | absent | absent | left absent | present | present | present | absent | absent | |
| Retinopathy | — | ++ | — | + | + | — | + | — | |
| Intercapillary glomerulosclerosis by renal biopsy | absent | membranous | absent | absent | absent | membranous | membranous | absent | |
| Pyelonephritis | — | — | — | + | + | ++ | ++ | — | |
| Urinary infection | | | | | | | | | |
| Previous | 0 | 1 | 0 | 0 | 0 | 2 | 2 | 1 | |
| Current | 1 | 0 | 0 | 1 | 1 | 2 | 2 | 1 | |
| Previous pregnancies | 0 | 0 | 1 | 1 | 1 | 7 | 1 | 3 | 1.6 |
| Living children | 0 | 0 | 1 | 1 | 0 | 3 | 1 | 1 | |
| Abortions | 0 | 0 | 0 | 0 | 1 | 4 | 0 | 1 | |
| Neonatal fetal death | | | | | | | | | |
| Current | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | |
| Toxemia of pregnancy | | | | | | | | | |
| Previous | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | |
| Current | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | |

*Hyperemesis gravidarum throughout.
†Studies made during two pregnancies.
‡Polyhydramnios during first pregnancy.
§Number of episodes requiring hospitalization.

## RESULTS

*Glomerular Filtration Rate and Renal Plasma Flow.* As a basis of comparing the findings in the individual patients, their values are superimposed in figure 1 upon the findings from a previous study of twelve normal pregnant women and nine controls. The central horizontal lines represent the mean of clearance values in normal pregnant controls, and the bars represent two standard deviations above and below the mean. Solid circles represent values from the diabetic subjects and "x"s in second pregnancies. It is evident that all but one (case 4) of the sets of clearance values fall within or above the normal range. In figure 2 is shown the mean and standard deviation of the baseline clearance studies done when the patients were not pregnant [six postpartum and one (case 8) before pregnancy]. The mean PAH clearance is reduced and the mean BUN elevated in contrast to that of the controls, but these differences are not significant in view of the wide individual variation. In figure 3 are shown the values during pregnancy. The control values are necessarily an approximation, since there is variation at different stages of pregnancy, and no single average is truly representative. In the diabetic subjects there is a proportionally greater increase in renal plasma flow with a resulting fall in filtration fraction, but, again, these changes are not statistically significant.

*Plasma Urea Nitrogen.* The plasma urea nitrogen concentrations measured in five of the diabetic patients when not pregnant were above the normal mean (figure 2). During pregnancy the values were considerably above the lowered mean which is normal for pregnancy (figure 3). These relative elevations were noted in spite of the fact that the filtration rates were only slightly reduced.

*Intercapillary Glomerulosclerosis.* Three patients (cases 2, 6 and 7) with duration of diabetes of 17, 13 and 13 years respectively, had on renal biopsy findings that would be classified according to Gellman's criteria[6] as "two plus" diffuse diabetic glomerulosclerosis as shown in figure 1. All three at various times had filtration rates and plasma flow above the normal mean. The one distinctive feature, however, was a filtration fraction at all times either below the normal range for pregnancy or in the lower range (from 0.11 to 0.18). Case 2, whose findings have been reported in detail elsewhere,[7] had a rise in filtration fraction which was maintained after delivery, and other features to suggest improve-

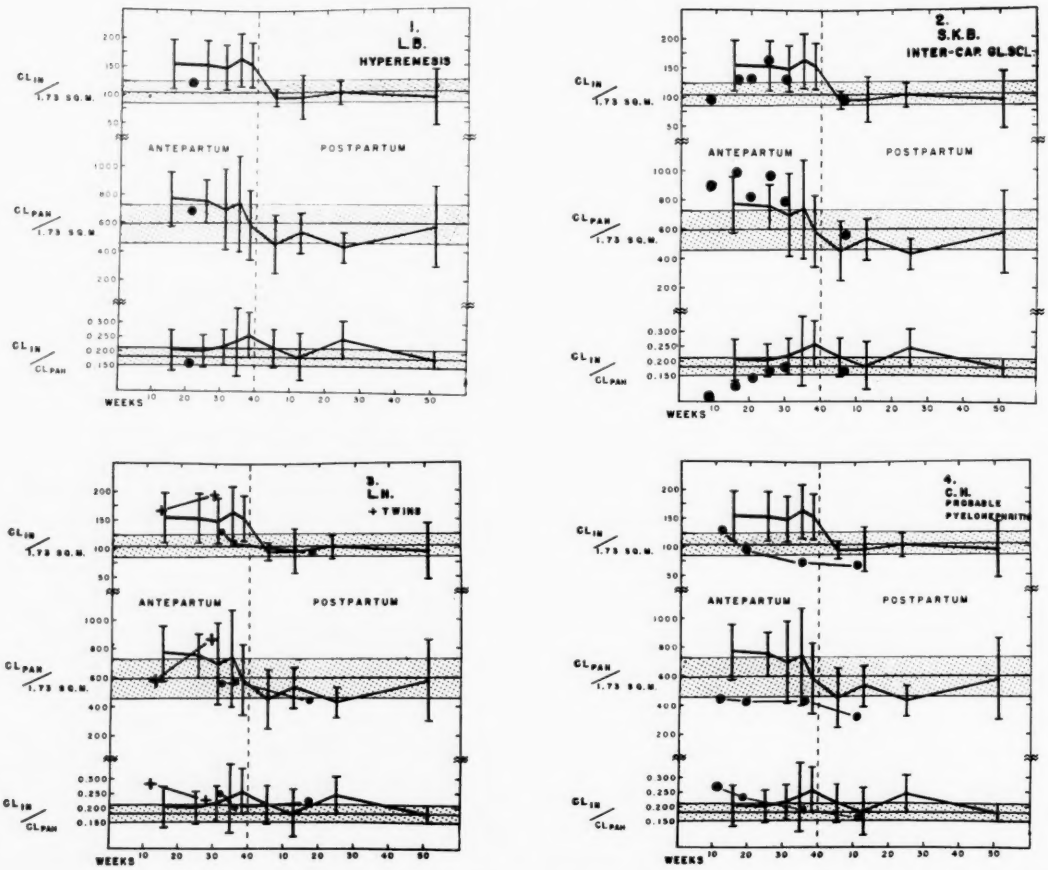



FIG. 1. Renal clearance values in individual diabetic patients during pregnancy shown in relation to the mean of

ment in renal and vascular status. The other two patients had less marked changes in the same direction. Two additional patients with diabetes of long duration (case 5 of seventeen years' duration, and case 8 of twenty-three years' duration) had minimal retinal microaneurysms and presumably had glomerulosclerosis in some degree, although this was not established by renal biopsy. Both of these patients had filtration rates in their second pregnancies which were reduced (0.14 to 0.16) and both pregnancies terminated in neonatal deaths from hyaline membrane disease.

*Pyelonephritis.* Three of the patients had clinical, bacteriological, and X-ray evidence of pyelonephritis (cases 5, 6 and 7), and one had probable pyelonephritis (case 4). Two of these (cases 6 and 7) also had diffuse glomerulosclerosis on renal biopsy, and are discussed above, but in spite of this had clearances well above the normal mean for pregnancy, while the other

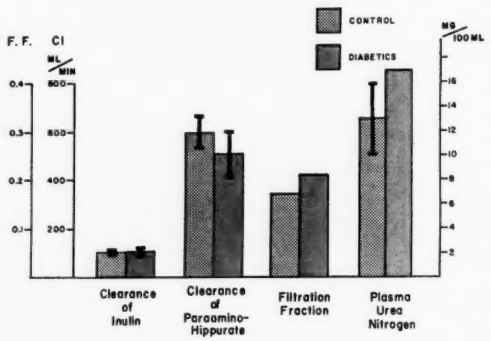
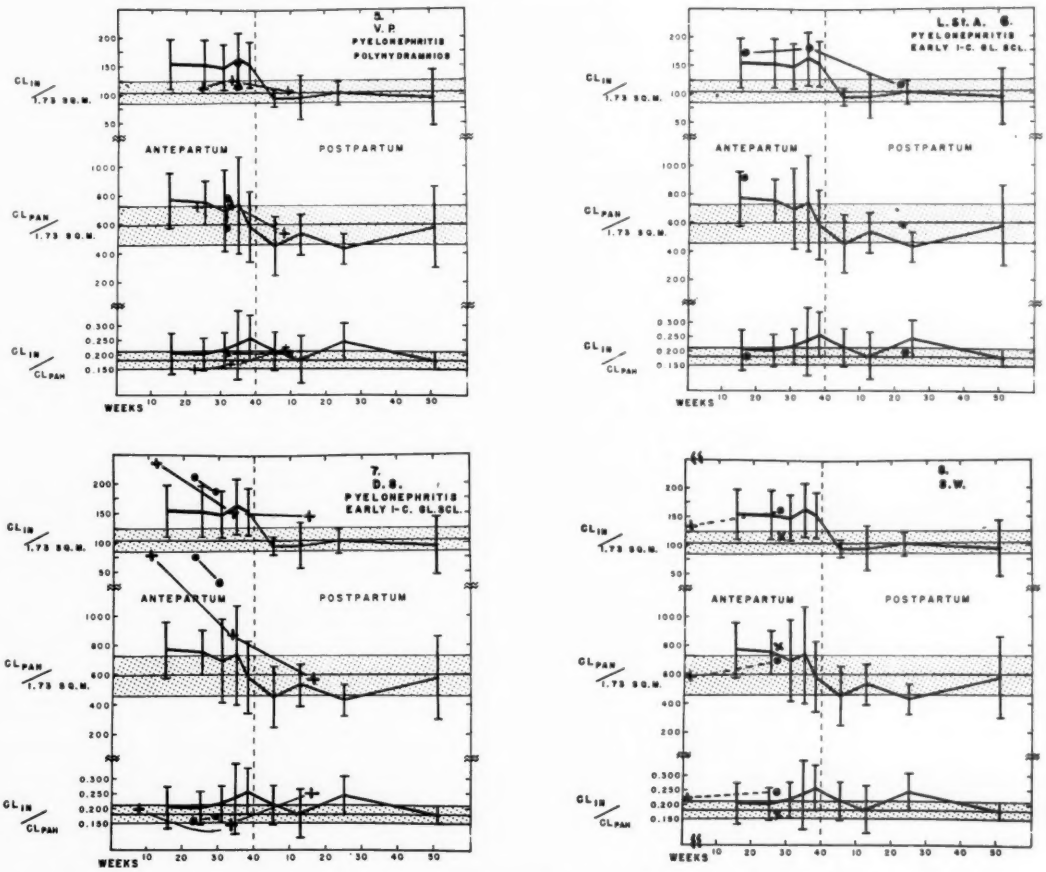



FIG. 2. Renal function in nine control subjects and in seven of the eight subjects of the present study when not pregnant. Bars represent the mean and one standard deviation.




normal pregnant and nonpregnant subjects. Solid circles represent values during first pregnancies and X's those in the second.*

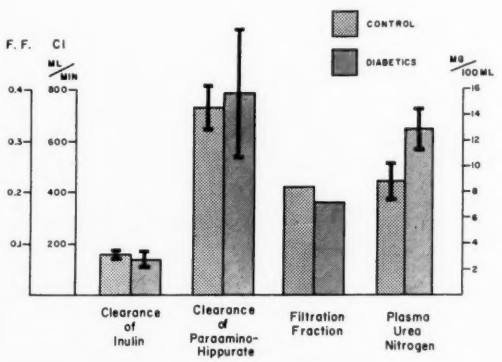



FIG. 3. Renal function in twelve control subjects and in eight diabetic subjects during pregnancy. The bars represent the mean and one standard deviation.

two cases (4 and 5), had values below the mean. One patient (case 4) had had a toxemia of pregnancy at the end of her first pregnancy and had a urinary infection early in her second pregnancy. Hers were the only clearance values which were consistently below the extreme normal range for pregnant subjects. Inasmuch as the filtration fraction declined progressively, and in view of the fact that her diabetes was of eleven years' duration, the possibility that she had progressive glomerulosclerosis cannot be excluded.

*Relation of Renal Clearances to Development of Edema.* In three patients (cases 3, 5 and 7) kidney function studies during five pregnancies were carried out at the time that moderate to marked physiological edema developed. One of the patients (case 3) had mild toxemia, with elevation of blood pressure and proteinuria as

* Reprinted through courtesy of *Annals of Internal Medicine* 53:4, April 1960.

well. Two of the three had normal clearances at these times as is shown in figure 1. One patient, who also had polyhydramnios, had reduced clearance when measurement was made in the supine position, and normal in the left lateral position (week 30, case 5, figure 5). On the other hand the patient with the most reduced renal function (case 4) did not have edema at any time during her pregnancy. In summary, there was no correlation between development of edema and filtration rate or renal plasma flow in this small series.

*Tubular Reabsorption of Glucose.* In figure 4A is illustrated the regression of maximal tubular reabsorption of glucose ($TM_G$) on glomerular filtration rate (GFR) taken from a series of normal pregnant subjects and controls previously studied in this laboratory.[4] It is apparent that there is a proportionate increase in $Tm_G$ and GFR in both the control and pregnant subjects. In figure 4B is graphed the tubular reabsorption of glucose ($T_G$) in glucosuria during a given study. The open circles indicate measurements during pregnancy. The ratio of filtered to reabsorbed glucose averaged only 1.32, and ranged from 1.1 to 2.6, so that tubular saturation was not achieved in a majority of cases, and the figures are not entirely comparable to the normal controls. The average of the $T_G$ during pregnancy for the eight subjects (277 mg./min.) was not significantly different from the average $T_G$ for five of the subjects measured in the nonpregnant state (268 mg./min.). However, the same relationships are apparent as in the study of pregnant subjects in which $Tm_G$ was measured. Once again, elevated filtration rates are usually associated with high $Tm_G$s, indicating a proportionally large functional renal mass. Because of this fact, those patients with higher filtration rates do not necessarily have an increase in the tendency toward glycosuria.

## DISCUSSION

To the present time we are aware of no studies of kidney function in pregnancy complicated by diabetes. A number of studies have been made of kidney function in diabetes complicated by intercapillary glomerulosclerosis with somewhat discrepant results. In a series of thirteen patients with various stages of the complication, Brun[8] found only one had reduced filtration fractions, and this patient had diffuse lesions on renal biopsy. On the other hand, all of the ten patients studied by Bucht[9] had reduced filtration fractions. During the current work it was noted that in diabetics Schiff-base combination of PAH with glucose in urine may give falsely low values for clearance of PAH. Possibly this interference may explain some of these discrepancies.

In the present study, which includes a small number of patients with proven diffuse intercapillary glomerulosclerosis, and several with this presumptive diagnosis, a

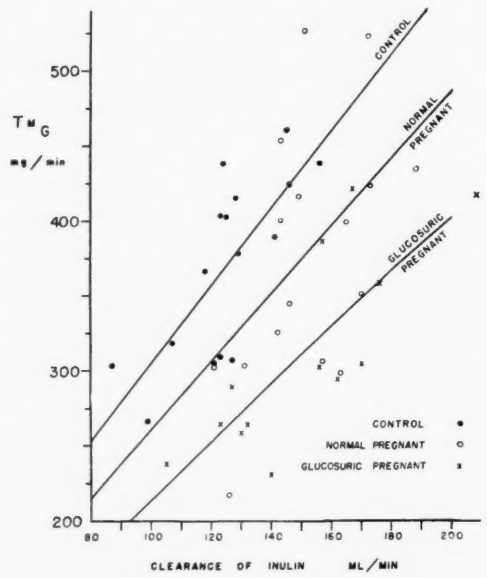



FIG. 4A. The regression of the maximal tubular reabsorption of glucose (TmG) on glomerular filtration rate in normal pregnant and control subjects.

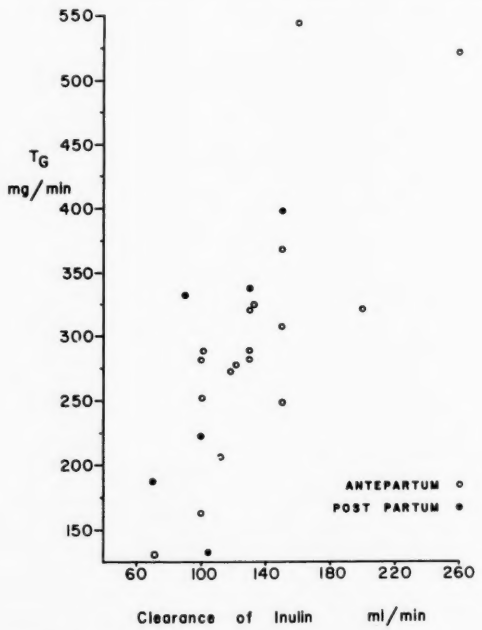



FIG. 4B. The regression of tubular reabsorption of glucose (TG) in diabetic subjects during pregnancy and postpartum.

low filtration fraction is a consistent finding. It is probable that all the patients have some degree of glomerular changes, since it has been demonstrated by electron microscopy that patients with diabetes of very recent duration may have thickening of the basement membrane. In one patient of this series, previously reported in detail,[4] there was apparent improvement in renal function and in retinopathy during and following pregnancy; in other patients of this series there was not this same suggestion of improvement during pregnancy. One patient had a falling filtration fraction during pregnancy and postpartum, and two other long-standing diabetics had lower filtration fractions during a second pregnancy and developed microaneurysms during the course of pregnancy. A constellation of factors must influence the progression or regression of lesions of small vessels, and there is presumably much individual variation in their relative effects.

Four of these patients had had pyelonephritis on the basis of clinical or roentgenological evidence. Kidney function studies per se were not of value in their diagnosis. In two patients biopsy demonstrated that intercapillary glomerulosclerosis was coexistent. The high incidence of bacilluria and urinary infection in pregnancy complicated by diabetes is borne out by this study. Seven patients had significant urinary infection at some time during the course of twelve pregnancies. During the latter part of the study Mandelamine with methionine in quantities sufficient to acidify the urine was given routinely after the manner of Kass.[10]

It was apparent in the present study that edema could develop in the face of normal or elevated filtration rates or renal plasma flows, measured in the conventional manner. One explanation for this apparent discrepancy may lie in the fact that when renal functions are measured in the reclining position, they do not necessarily reflect the function during a greater portion of the day when the patient is ambulatory. Dignam and Assali[11] have shown that in normal pregnant subjects, quiet standing produces an exaggerated reduction of filtration rate, or renal plasma flow, and of urine flow, as well as an increase in aldosterone production, presumably as a result of translocation of body fluids and also of alteration of renal blood supply by the gravid uterus. In the diabetic patient with polyhydramnios the contrast in function between the lateral and the supine position was striking. Presumably the reduction in secretion of water is the result of a reduction of free water reaching the distal and collecting tubule secondary to reduction of filtration, since physiological edema and preeclampsia may develop in patients with diabetes insipidus.[12] The importance of postural effects on the redistribution of body fluids on standing may be even greater in patients with diabetes of long standing, due to the increased incidence of neuropathy in these patients. Sharpey-Schaeffer[13] has recently demonstrated an absence of vascular reflexes in such patients. Although no data on this score are available, resulting orthostatic hypotension might be expected to reduce renal blood flow and filtration rate. In this series four of the patients had some evidence of neuropathy.

During normal pregnancy the range for blood urea nitrogen is sharply reduced so that the values all lie below the normal mean for nonpregnant women. Although the filtration rates were not relatively reduced in the pregnant diabetic subjects, the plasma urea nitrogen concentrations were increased beyond the normal range. A higher intake of protein and increased catabolism of proteins may in part explain the increase. Likewise the fact that the filtration rate may be relatively more reduced in these subjects when ambulatory may also explain the discrepancy.

From a clinical standpoint it is important to know whether the so-called renal threshold for glucose is lowered in those diabetic subjects who develop an increase in filtration rate, and hence in the filtered load of glucose during pregnancy. This will depend on two factors: (1) whether the tubular capacity to reabsorb glucose is proportionate to the increase in filtered load, and (2) whether there is an actual lowering of the capacity of the renal tubules to reabsorb glucose ($Tm_G$) during pregnancy. Smith[14] in 1943 showed that in normal women the $Tm_G$ was in fact proportionate to the filtration rate. In a recent study by Welsh[4] in this laboratory of the mechanism of renal glycosuria of pregnancy this same relationship was found in pregnant subjects. The $Tm_G$ was not significantly lower in pregnant than in nonpregnant women. Patients with glucosuria had lower $Tm_G$s than the nonglucosuric women. Patients with glucosuria had lower $Tm_G$s than the nonglucosuric subjects, but evidence is not available that these are actually *lowered* by pregnancy. There was a relative preponderance of filtered load of glucose over the capacity to reabsorb, in both normal and glucosuric subjects, in that the ratio of $GFR/Tm_G$ was higher. Thus all of the pregnant subjects had an increased tendency to develop glucosuria, and those subjects with a low $Tm_G$ did so.

In the present study of diabetic subjects the tubular reabsorption of glucose during periods of glucosuria had the same correlation noted originally by Smith; i.e., elevated filtration rates were in general associated

with greater capacity of the tubules to reabsorb glucose. The values of $T_G$ during pregnancy cannot be directly compared with the values measured when the subjects were not pregnant, since $Tm_G$ was not measured. If there develops a preponderance of filtered load over capacity to reabsorb as a result of the increased filtration in the diabetic as in the normal, which seems likely, then the renal threshold of diabetics should be somewhat lowered in pregnancy. That this is actually the case is suggested by the studies of Monicke and Worm,[15] who demonstrated a lowered threshold for glucose as pregnancy progresses.

## SUMMARY

1. Renal clearance studies and measurement of the tubular reabsorption of glucose have been carried out during and after twelve pregnancies in eight long-standing diabetics.

2. Essentially the same increase in filtration and in renal plasma flow was noted in this group of patients as in normal pregnant subjects. There was, however, a proportionally greater increase in plasma flow than in filtration rate so that the filtration fraction was lower than in most normal subjects.

3. The characteristic finding among patients whose pregnancy was complicated by intercapillary glomerulosclerosis was a reduced filtration fraction.

4. In diabetic pregnancy complicated by pyelonephritis, renal clearances may be normal or above normal and are not diagnostic.

5. Physiological edema of pregnancy in these subjects developed at times in association with a glomerular filtration rate normal for the stage of pregnancy.

6. The tendency toward glucosuria is increased as a result of the increase in filtration rate.

## SUMMARIO IN INTERLINGUA

*Studios Serial del Function Renal in Pregnantia*

1. Studios del clearance renal e mesurationes del reabsorption tubular de glucosa esseva effectuate durante e post dece-duo pregnantias in octo diabeticas veteran.

2. Essentialmente le mesme acceleration del filtration e del fluxo de plasma renal esseva notate in iste gruppo de patientes como in normal subjectos pregnante. Tamen, il occurreva in le patientes un proportionalmente plus marcate acceleration del fluxo de plasma que del filtration, de maniera que le fraction filtrational esseva plus micre que in le majoritate del subjectos normal.

3. Un reduction del fraction filtrational esseva le constatation characteristic in patientes in qui le pregnantia esseva complicate per glomerulosclerosis intercapillar.

4. In pregnantias diabetic que es complicate per pyelonephritis, le clearance renal pote esser normal o supranormal e non es de fortia diagnostic.

5. Edema physiologic de pregnantia se disveloppava in iste subjectos a vices in association con un filtration glomerular de intensitate normal pro le stadio de pregnantia in question.

6. Le tendentia glucosurial es potentiate como resultato del accelerate filtration.

## ACKNOWLEDGMENT

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## *Biochemical Individuality*

Biochemical individuality is the possession of biochemical distinctiveness by individual members of a species, whether plant, animal or human. The primary interest in such distinctiveness has centered in the human family, and in the distinctiveness within animal species as it might throw light on human biochemistry.

While it has been known for centuries that bloodhounds, for example, can tell individuals apart even by the attenuated odors from their bodies left on a trail, the first scientific work which hinted at the existence of substantial biochemical distinctiveness in human specimens was the discovery of blood groups by Landsteiner about 1900.

A few years later Garrod noted what he called "inborn errors of metabolism"—rare instances where individuals gave evidence of being abnormal biochemically in that they were albinos (lack of ability to produce pigment in skin, hair and eyes), or excreted some unusual substance in the urine or feces. To Garrod these observations suggested the possibility that the biochemistry of all individuals might be distinctive.

About fifty years later serious attention to the phenomenon of biochemical individuality resulted in the publication of several articles and a book on this subject. These reported evidence indicating that every human being, including all those designated as "normal," possesses a distinctive metabolic pattern which encompasses everything chemical that takes place in his or her body. That these patterns, like the abnormalities discussed by Garrod, have genetic roots is indicated by the pioneer explorations of Beadle and Tatum in the field of biochemical genetics in which they established the fact that the potentiality for producing enzymes resides in the genes.

Biochemical individuality, which is genetically determined, is accompanied by and in a sense based upon anatomical individuality, which must also have a genetic origin. Substantial differences, often of large magnitude, exist between the digestive tracts, the muscular systems, the circulatory systems, the skeletal systems, the nervous systems, and the endocrine systems of so-called normal people. Similar distinctiveness is observed at the microscopic level, for example in the size, shape and distribution of neurons in the brain and in the morphological "blood pictures," i.e., the numbers of different types of cells in the blood.

Individuality in the biochemical realm is exhibited with respect to (1) the composition of blood, tissues, urine, digestive juices, cerebrospinal fluid, etc.; (2) the enzyme levels in tissues and in body fluids, particularly the blood; (3) the pharmacological responses to numerous specific drugs; (4) the quantitative needs for specific nutrients—minerals, amino acids, vitamins—and in miscellaneous other ways including reactions of taste and smell and the effects of heat, cold, electricity, etc. Each individual must possess a highly distinctive pattern, since the differences between individuals with respect to the measurable items in a potentially long list are by no means trifling. Often a specific value derived from one "normal" individual of a group will be several times as large as that derived from another.

The implications of this individuality are extremely broad. For medicine they suggest that susceptibility to all disease—infective, metabolic, degenerative, mental or unclassifiable (including cancer) probably has its roots in biochemical individuality and that the differences in responses to drugs including alcohol, caffeine, nicotine, carcinogens and morphine derivatives, which are well authenticated, have a sound and discoverable basis. It is a well-known fact that conditions which will produce disease in certain individuals will not do so in others. The basis for this observation has hitherto not been recognized; it doubtless has its roots in biochemical individuality—a development which has received little attention. People definitely possess what may be called "biochemical personalities" and it seems extremely likely that these are meaningful in connection with the numerous and increasing personality disorders and difficulties which afflict men and women in modern life.

By Roger J. Williams, in *Encyclopedia of Biological Sciences,* edited by Dr. Peter Gray, Head, Department of Biology, University of Pittsburgh, Reinhold Publishing Corp., New York, 1961.

# An Evaluation of the Pooled Diaphragm Technic for Estimating Insulin Activity

*G. S. Wiberg, Ph.D., and D. C. Jessup, Ph.D., Ottawa*

Following the original observation of Gemmill[1,2] that insulin increases the glucose uptake of isolated rat diaphragm, a number of methods have been designed for the estimation of insulin activity which utilize this response.[3-7] However, large differences in the basal glucose uptake between individual diaphragms and variations in response of the tissues of different rats to the same dose of insulin tend to lower the precision of this type of assay.

A possible procedure for reducing this inherent variability is to increase the number of responses in each dose group. Obviously this expedient has limited application to in vitro technics. An alternative plan is to use pooled hemidiaphragms from different animals, i.e., a number of tissues incubated together in the same flask. Presumably this pooling would minimize the individual variation and thus reduce the standard error of the estimate.

The technics described by Groen, Kamminga, Willebrands and Blickman,[3] and Takeuchi, Ohashi and Kobayashi,[5] each measure the glucose uptake from four pooled hemidiaphragms from different rats. Oyama and Grant[7] determined the excess glucose uptake by five pooled mouse hemidiaphragms in the presence of insulin over that obtained with the corresponding untreated hemidiaphragms.

In addition the latter workers have studied various factors affecting glucose uptake and believe the method employing mouse hemidiaphragms is an improvement over the rat diaphragm technic from the standpoint of reliability and reproducibility.[7]

In the present investigation, a modification of the method described by Oyama and Grant[7] was followed to study the relation between insulin concentration and glucose uptake by mouse hemidiaphragms. A statistical approach has been used which permits estimation of both the potency and the limits of error. In addition, a possible reason for the variability encountered with this type of assay has been investigated.

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## METHODS

Male mice, weighing between 25 to 30 gm., of an inbred strain procured from a colony reared in the Food and Drug Directorate were used. These were fasted sixteen to twenty-four hours, killed by decapitation, the diaphragms removed and treated in accordance with the technic described by Oyama and Grant.[7] Glucose utilization was estimated in the respective media, after deproteinization with barium hydroxide-zinc sulfate,[8] by the anthrone method.[9] Five pooled hemidiaphragms were incubated with graded dose levels of insulin (prepared from Zinc Insulin Crystals—Connaught Medical Research Laboratories) while the corresponding five hemidiaphragms were incubated without insulin. Excess glucose uptake was determined by difference in the utilization of glucose between treated and control tissues and was expressed as milligrams glucose per 10 mg. dry weight per ninety minutes. Individual assays of the balanced four point design[10] were performed and the results analyzed according to the procedures described by Bliss and Marks.[11]

## RESULTS

(a) *Dose response relationship.* A positive response could be elicited with as little as 10 to 20 *u*U of insulin. The values obtained in eleven consecutive determinations in which the four different dose levels were measured each day are given in table 1. A num-

TABLE 1

Effect of insulin on the glucose uptake of pooled mouse hemidiaphragms

| Insulin dosage in micro-units | Number of results | Excess glucose uptake (mg./10 mg. dry wt./90 minutes | Standard error | Range (mg./10 mg. dry wt./90 minutes |
|---|---|---|---|---|
| 50 | 8 | 0.054 | ±0.125 | 0.02—0.12 |
| 500 | 10 | 0.148 | ±0.036 | 0.03—0.28 |
| 5,000 | 10 | 0.209 | ±0.036 | 0.08—0.44 |
| 50,000 | 9 | 0.286 | ±0.078 | 0.02—0.68 |

ber of negative results (i.e., greater glucose uptake in controls than in treated tissues) occurred which have not been included in the present study. This accounts for the discrepancy between the results recorded and the number of experiments performed.

Even though the log-dose interval was 1.0, the differences between succeeding doses were not significant. The range at each dose level increased in direct proportion to the glucose uptake thus providing extensive overlap in the response to insulin.

Although there is a relation between the log dose of insulin and excess glucose uptake, a significant difference between the mean responses does not become apparent until a log-dose interval of 2.0 is attained. These observations indicate that a standard log-dose response curve should not be employed for estimating insulin activity. In order to overcome this difficulty, a balanced four point design was employed using two estimates of the glucose at each dose level for both reference standard and unknown. The results of five assays in which the same sample of commercial insulin was used as both standard and unknown are shown in table 2. The log-dose interval was 1.0 throughout.

Analyses of variance indicated that the first three assays were valid. The potency ratios (M) varied considerably although they did not differ significantly. The slope in Assay No. 3 was much lower than in Assay No. 1 or 2. Assay No. 4 could not be used because of negative uptake at low dose level for the unknown. In Assay No. 5 the dose response lines were not parallel. In order to measure the potency with greater precision, it would be necessary to combine several individual assays.

*Glucose uptake by untreated tissues.* The presence of inactive tissue in the pooled hemidiaphragms might explain this wide variation in excess glucose uptake for a given level of insulin. Such tissues could not be detected but would cause an unsatisfactory base line. This hypothesis was investigated by comparing the control levels of glucose uptake by sets of five left hemidiaphragms with those of the corresponding right hemidiaphragms. The results obtained for eighteen such comparisons, six each on three separate days, are given in table 3.

Under ideal conditions, the difference in glucose uptake between corresponding halves of the pooled tis-

TABLE 2

Insulin assays using pooled mouse hemidiaphragms and balanced four point design

| Assay number | Dosage* | Response† Standard | Response† Unknown | b | s | λ | Relative potency of unknown Per cent | P = 0.95 Range of potency Per cent | P = 0.95 Limits of error Per cent |
|---|---|---|---|---|---|---|---|---|---|
| | 50 | .07 | .07 | | | | | | |
| | | .07 | .12 | | | | | | |
| 1 | | | | .193 | .002 | .090 | 109 | 73-164 | 67-150 |
| | 500 | .28 | .27 | | | | | | |
| | | .28 | .27 | | | | | | |
| | 500 | .32 | .32 | | | | | | |
| | | .26 | .20 | | | | | | |
| 2 | | | | .228 | .060 | .263 | 154 | 47-505 | 30-328 |
| | 5,000 | .45 | .51 | | | | | | |
| | | .44 | .61 | | | | | | |
| | 50 | .02 | .02 | | | | | | |
| | | .04 | .02 | | | | | | |
| 3 | | | | .053 | .020 | .377 | 24 | 4-134 | 18-548 |
| | 500 | .13 | .05 | | | | | | |
| | | .08 | .05 | | | | | | |
| | 50 | .07 | —.02 | | | | | | |
| | | .01 | —.12 | | | | | | |
| 4 | | | | | | negative uptake—assay not valid | | | |
| | 500 | .08 | .02 | | | | | | |
| | | .04 | .17 | | | | | | |
| | 500 | .22 | .04 | | | | | | |
| | | .24 | .01 | | | | | | |
| 5 | | | | | | dose response lines—not parallel assay not valid. | | | |
| | 5,000 | .36 | .36 | | | | | | |
| | | .29 | .45 | | | | | | |

*Microunits of insulin per milliliter.
†Excess glucose uptake in mg./10 mg. dry weight/90 minutes.

sues should approach zero. However, this was seldom achieved in the present study. In only two of the eighteen trials did the right and left hemidiaphragms show the same glucose uptake. In five sets of hemidiaphragms, one of the pools did not remove glucose from the medium. In one trial, neither pool displayed glucose uptake. If only a portion of the tissues in any flask was responsive, a reduction in total uptake would be expected. This possibility would make the differences in glucose utilization between corresponding pools greater than the theoretical value of zero. The results shown in table 3 strongly suggest that variations can be attributed to the presence of nonresponsive hemidiaphragms in the individual pools.

TABLE 3

Glucose uptake by untreated, pooled, paired hemidiaphragms

| Day | Total glucose uptake—mg. per 10 mg. dry wt. per 90 minutes: Left hemidiaphragms | Right hemidiaphragms | Difference |
|---|---|---|---|
| 1 | 0.64 | 0.55 | 0.09 |
| | 0.44 | 0.22 | 0.22 |
| | 0.00 | 0.29 | 0.29 |
| | 0.70 | 0.13 | 0.57 |
| | 1.19 | 0.66 | 0.43 |
| | 0.21 | 0.00 | 0.21 |
| 2 | 0.57 | 0.49 | 0.08 |
| | 0.32 | 0.47 | 0.15 |
| | 0.00 | 0.10 | 0.10 |
| | 0.29 | 0.29 | 0.00 |
| | 0.21 | 0.87 | 0.66 |
| | 0.31 | 0.00 | 0.31 |
| 3 | 0.42 | 0.31 | 0.11 |
| | 0.14 | 0.08 | 0.06 |
| | 0.10 | 0.10 | 0.00 |
| | 0.08 | 0.22 | 0.14 |
| | 0.00 | 0.00 | 0.00 |
| | 0.00 | 0.10 | 0.10 |

## DISCUSSION

A principal difficulty encountered in this investigation was the impossibility of assessing the viability of individual tissues in each group of five hemidiaphragms. The fact that negative glucose uptake was found on occasion and that a considerable difference in uptake occurred between corresponding halves of the same diaphragm (table 3) suggested a variation in the degree of activity of the tissues. It is well known that inactive tissues may occur in in vitro preparations. Since insulin activity is measured by the difference between treated and control pools of hemidiaphragms, the presence of a nonviable tissue in either pool can lead to drastic alteration in the criterion of response. Thus the validity of individual responses is always questionable.

## SUMMARY

Consideration has been given to the use of pooled mouse hemidiaphragms for the in vitro bio-assay of insulin. The occurrence of negative values in some instances indicated that it would be impossible to differentiate between a true insulin response and a spurious difference resulting from the presence of nonviable or inactive tissue in the pool.

## SUMMARIO IN INTERLINGUA

*Un Evalutation del Technica de Collectas de Diaphragma pro Estimar le Activitate de Insulina*

Ha essite considerate le uso de collectas de diaphragma murin pro le bio-essayage in vitro de insulina. Le occurrentia de valores negative in certe casos indicava que il esserea impossibile differentiar inter un ver responsa insulinic e un valor spurie resultante del presentia de tissu non-viabile o inactive in le collecta.

## ACKNOWLEDGMENT

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# Insulin Bio-assay Using Glycogen Deposition in a Single Rat Diaphragm

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Numerous in vitro assay procedures for insulin exist which utilize glucose uptake by the isolated rat diaphragm[5,11,16,17] or by rat adipose tissue[1,8] as the criterion of response. There are certain features of these technics which tend to affect the precision and reliability of these assays. These include: (a) large differences in glucose uptake between individual rat diaphragms, (b) variation in response of the tissues of different rats to the same dose of insulin[8,21] and (c) the day-to-day variation in basal glucose uptake and in the extra glucose uptake due to insulin which can be significant.[7] An extensive and critical review of the work in this field has been published recently by Vallance-Owen and Wright.[18]

In order to circumvent these difficulties, certain workers have resorted to pooling diaphragms from either rats[5,16] or mice.[9] However, it has been shown[20] that pooling of hemidiaphragms of mice tends to introduce an additional source of error due to the possible presence of nonviable sections either in the control tissues or in the insulin treated tissues.

Cosma[4] in his investigations of the effect of thyroxine on glucose uptake in rat diaphragm eliminated the between-animals variation by using seven sections of the same tissue for his observations. It appeared of interest to apply this approach to the in vitro assay of insulin to determine if it would increase the precision of the assay.

Glycogen synthesis in muscle is a well-established response to insulin.[18] Preliminary studies indicated that glycogen deposition in the rat diaphragm is less variable than glucose uptake. This suggested that the amount of extra glycogen deposited in different sections of the same rat diaphragm might serve as a suitable response metameter for the in vitro bio-assay of insulin. Perlmutter, Weisenfeld and Mufson[10] used glycogen deposition in isolated rat diaphragm as the response criterion for insulin assay but reported only limited success with this technic.

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## METHODS

Male and female rats of an inbred Wistar strain, weighing 150-200 gm., were used. These animals were fed a standard laboratory chow and were not fasted before use. For each assay, a single rat was sacrificed by decapitation and the diaphragm was quickly excised and rinsed in ice-cold Krebs-Ringer-bicarbonate (K.R.B.) solution. The central tendinous section and any extraneous fat were removed before dividing the diaphragm into eight approximately equal sections. Each portion was immediately placed in a separate Warburg flask containing 1.0 ml. Krebs-Ringer-bicarbonate solution plus glucose (2.0 mg. per milliliter). Various insulin concentrations were present in appropriate flasks as required. The flasks were gassed for ten minutes with 95 per cent oxygen and 5 per cent carbon dioxide, then incubated for ninety minutes at 38° C. at a shaking rate of 100 cycles per minute.

After incubation, the glucose concentration of the medium and the glycogen concentration in the tissues were determined. Glucose was measured using the anthrone reagent[13] on the diluted medium which had been deproteinized with barium hydroxide-zinc sulphate.[14] The tissue uptake of glucose was taken as the difference between the initial and final concentrations of glucose in the medium and this was expressed as milligrams glucose per gram wet tissue per ninety minutes.

"Free" glycogen[6] was determined by a modification of the liver glycogen method described by van der Vies.[19] Tissues were individually blotted dry, weighed and placed in 2.0 ml. of 5 per cent trichloracetic acid. They were then homogenized in a Potter-Elvehjem glass homogenizer and the homogenate centrifuged. One-milliliter aliquots of the supernatant solution were added to equal volumes of 10 N KOH and heated in a boiling water bath for sixty minutes. It was found that this procedure destroyed 99 per cent of the reducing sugar while causing a loss of less than 2 per cent of the glycogen. The tubes were cooled, the contents neutralized with glacial acetic acid and diluted to 5.0 ml. with distilled water.

Glycogen determinations were carried out on aliquots of these dilutions, using the anthrone reagent. The results were expressed as milligrams glycogen per gram of wet tissue per ninety minutes.

Choice of the above technic for determining glycogen was made to avoid measuring the fixed glycogen which, being unresponsive to insulin, would only increase the error. Kits van Heijningen[8] has shown that only "free" glycogen is affected by insulin and this can be extracted with 5 per cent trichloracetic acid at room temperature.

## RESULTS

A number of preliminary experiments were carried out to determine the effects of tissue size, variation between animals and variation between sections of the same diaphragm. The dose response relation was studied and a number of bio-assays were completed.

*Effects of tissue size*

Several investigations have indicated that the size of the isolated tissue affects the response to insulin.[5,9,17] An experiment was designed to determine the effect of tissue size on glucose uptake and glycogen deposition of sections taken from the same diaphragm.

The diaphragms were removed from six rats on different days and were sectioned to provide a hemi-diaphragm, a quarter-diaphragm and two eighth-diaphragms from each animal. The sections were incubated separately with K.R.B.-glucose containing insulin (500 *u*U per milliliter). The volume of the incubating medium in each case was 1.0 ml. per eighth-diaphragm. Glycogen and glucose determinations were made for each section and the mean response for each tissue size was determined.

The results shown in table 1 indicate that no significant differences in glucose uptake or glycogen deposition ensue from employing tissues of different size when they are taken from the same animal ($P>0.1$).

TABLE 1

Effect of tissue size on glucose uptake and glycogen deposition by sections of isolated rat diaphragm

| Size of section | No. of observations | Tissue weight (mg.) | Glucose uptake mg./gm. wet weight/ninety minutes | Glycogen synthesis mg./gm. wet weight/ninety minutes |
|---|---|---|---|---|
| Hemi-diaphragm | 6 | 144.4±6.74* | 11.5±1.26* | 4.82±0.91* |
| Quarter-diaphragm | 6 | 73.3±3.80 | 11.1±1.44 | 4.56±0.36 |
| Eighth-diaphragm | 12 | 32.0±1.01 | 11.4±1.33 | 4.09±0.42 |

*Standard error of the mean.

*Comparison of within-animals and between-animals variation*

(a) *Within animals*: Diaphragms were removed from five rats on different days and each diaphragm was divided into eight equal parts. Each section was incubated separately in the Krebs-Ringer-bicarbonate-glucose medium. Glucose and glycogen determinations were made as before. The mean responses for each diaphragm were calculated and these are presented in table 2.

(b) *Between animals*: Two sections of one-eighth diaphragm from ten different rats were incubated separately and the glucose uptake and glycogen deposition were determined. The mean responses for the twenty sections are given in table 2.

A comparison of the standard deviations for the mean responses obtained under the different conditions

TABLE 2

Comparison of within-animal and between-animals variation with respect to glucose uptake and glycogen deposition

| No. of tissues (eighth-diaphragms) | Glucose uptake mg./gm. wet tissue/ninety minutes | | | Glycogen deposition mg./gm. wet tissue/ninety minutes | | |
|---|---|---|---|---|---|---|
| | Mean | S.D. | S.E. | Mean | S.D. | S.E. |
| | | | A — within-animal | | | |
| 8 | 5.47 | 1.41 | 0.50 | 2.42 | 0.28 | 0.10 |
| 8 | 4.53 | 2.02 | 0.72 | 1.65 | 0.25 | 0.09 |
| 8 | 9.91 | 3.82 | 1.35 | 1.91 | 0.23 | 0.08 |
| 8 | 6.07 | 2.38 | 0.84 | 3.10 | 0.39 | 0.14 |
| 8 | 4.65 | 1.57 | 0.56 | 1.87 | 0.14 | 0.05 |
| | | | B — between-animals | | | |
| 20* | 5.71 | 6.00 | 1.35 | 2.80 | 0.98 | 0.22 |

S.D. = Standard deviation; S.E. = Standard error.
*This represents two eighth-diaphragms taken from ten different rats on different days.

demonstrates much greater variation between animals than exists within animals. Further, the variability of the glucose uptake was greater than that of glycogen deposition. Relative to their respective means, the standard deviation for glucose uptake measured on tissues from different rats on different days is ten times as great as that for glycogen synthesis measured in a single rat. Similarly it can be seen that glucose uptake by sections of a single diaphragm is a more variable response than glycogen deposition in the same tissue, i.e., as a proportion of their respective means the standard deviations for glucose uptake are approximately three times as great as the corresponding standard deviations for glycogen deposition.

*Between-animal and within-animal response to insulin*

In this experiment, each of six sections from one diaphragm was incubated in the Krebs-Ringer-bicarbonate-glucose solution with a single dose of insulin. The remaining two sections were incubated without insulin. Glucose uptake and glycogen deposition were determined and expressed as extra glucose uptake and extra glycogen deposition, i.e., the difference in response between control and treated tissues. In this manner six dose levels of insulin were assayed, each on a separate diaphragm. The results of these assays are presented in table 3a.

While no dose-response relation is evident from these results there is a definite effect of insulin on both response criteria. The lack of a dose-response relationship demonstrates the variation occurring between animals. The difference in sensitivity of rats to insulin is illustrated by the greater response occurring with lower dose levels than with certain higher doses. Again the internal variation between sections is slight as indicated by the standard errors of the means.

*Dose response relationship*

A series of twelve assays were performed to determine the relationship between the dose of insulin and both glucose uptake and glycogen deposition. In each experiment, six of the eight sections were treated separately with different dose levels of insulin ranging from 25 to 10,000 $\mu$U per milliliter. The remaining two sections served as controls. Extra glucose uptake and extra glycogen deposition were determined.

The results of these studies are shown in table 3b and graphically in figure 1. Analyses of variance (table 4) revealed that for both responses, the linear regression was highly significant ($P<0.01$) while the deviations from regression were not significant. Each response metameter, i.e., glucose uptake and glycogen deposition, displayed a significant between-animals variation, although the glycogen synthesis had a lower variance ratio. The equation for the regression line for glucose uptake was $Y = 2.54 \times -2.01$, where Y represents the glucose utilization in milligrams per gram of wet tissue per ninety minutes and X represents the log-dose of insulin in microunits per milliliter. The regression line for glycogen deposition was $Y = 2.14 \times -2.74$. The metameter, Y, represents the glycogen synthesis in milligrams per gram wet tissue per ninety minutes while X is the log-dose of insulin as described above.

TABLE 3

Effect of insulin on glucose uptake and glycogen deposition in sections of isolated rat diaphragm

| Insulin microunits per ml. | Number of determinations | Excess glucose uptake (mg./gm. tissue/ninety minutes) Mean | S.D. | S.E.M. | Excess glycogen deposition (mg./gm. tissue/ninety minutes) Mean | S.D. | S.E.M. |
|---|---|---|---|---|---|---|---|
| | | (a) Each dosage restricted to sections of the same diaphragm | | | | | |
| 25 | 6 | 2.32 | 1.79 | 0.73 | 0.53 | 0.13 | 0.08 |
| 100 | 6 | 8.65 | 4.22 | 1.72 | 1.85 | 0.29 | 0.12 |
| 250 | 6 | 6.66 | 3.34 | 1.36 | 1.68 | 0.12 | 0.05 |
| 1,000 | 6 | 5.51 | 1.64 | 0.67 | 2.84 | 0.46 | 0.19 |
| 2,500 | 6 | 9.43 | 2.40 | 0.98 | 1.83 | 0.24 | 0.10 |
| 10,000 | 6 | 7.12 | 2.48 | 1.01 | 5.87 | 0.54 | 0.22 |
| | | (b) Each dosage given to sections from different diaphragms | | | | | |
| 25 | 12 | 2.00 | 0.99 | 0.28 | 0.67 | 0.48 | 0.14 |
| 100 | 12 | 2.73 | 1.29 | 0.37 | 1.16 | 0.47 | 0.13 |
| 250 | 12 | 3.78 | 1.49 | 0.43 | 2.16 | 0.66 | 0.19 |
| 1,000 | 12 | 4.81 | 1.94 | 0.56 | 3.59 | 0.96 | 0.28 |
| 2,500 | 12 | 6.92 | 3.37 | 0.97 | 4.44 | 1.24 | 0.35 |
| 10,000 | 12 | 8.38 | 3.19 | 0.92 | 6.07 | 1.34 | 0.38 |

S.D. = Standard deviation.
S.E.M. = Standard error of the mean.

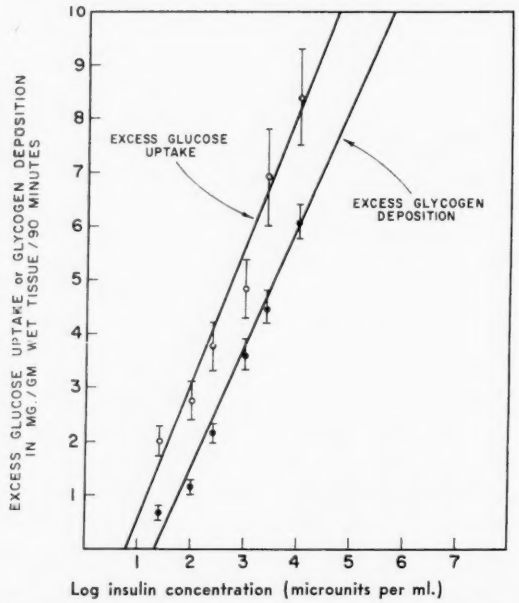
EXCESS GLUCOSE UPTAKE or GLYCOGEN DEPOSITION IN MG./GM WET TISSUE/90 MINUTES
EXCESS GLUCOSE UPTAKE
EXCESS GLYCOGEN DEPOSITION
Log insulin concentration (microunits per ml.)

FIG. 1. Dose response relation between insulin concentration and excess glucose uptake or excess glycogen deposition. Each value shown is the mean of twelve determinations plus or minus the standard error of the mean.

TABLE 4

Analysis of variance of response parameters for in vitro bio-assay of insulin.

| Glucose uptake Source | d.f. | s.s. | m.s. | F. | P. |
|---|---|---|---|---|---|
| Between doses | | | | | |
| (a) linear regression | 1 | 350.92 | 350.92 | 163.98 | 0.01 |
| (b) deviation from regression | 4 | 13.87 | 3.47 | 1.62 | |
| Between animals | 11 | 214.55 | 19.50 | 9.11 | 0.01 |
| Within doses (error) | 55 | 117.83 | 2.14 | | |
| | 71 | | | | |

s = 1.46 b = 2.54 (s/b) = 0.574

| Glycogen deposition Source | d.f. | s.s. | m.s. | F. | P. |
|---|---|---|---|---|---|
| Between doses | | | | | |
| (a) linear regression | 1 | 249.87 | 249.87 | 454.30 | 0.01 |
| (b) deviation from regression | 4 | 1.86 | 0.47 | 0.85 | |
| Between animals | 11 | 25.67 | 2.33 | 4.23 | 0.01 |
| Within doses (error) | 55 | 30.64 | 0.55 | | |
| | 71 | | | | |

s = 0.746 b = 2.14 (s/b) = 0.348

Symbols employed have the usual statistical meaning, i.e., d.f. = degrees of freedom; s.s. = sum of the squares; m.s. = mean square, (s.s./d.f.); F = variance ratio, (treatment variance/error variance); P = Probability; s = standard error, and b = slope of the log dose response line.

*Assay of commercial insulin*

To determine if the single diaphragm technic could be used with confidence to assay insulin, a series of experiments were made. Insulin which had previously been assayed independently in this laboratory by the mouse-sloping screen method of Stephenson[15] was used. Balanced four-point assays with two responses per dose level were employed. Insulin Toronto (Connaught Medical Research Laboratories—Toronto) was assayed against itself at two different dilutions and NPH Insulin (Connaught Medical Research Laboratories) was assayed against Insulin Toronto.

TABLE 5

Bio-assay of insulin by glucose uptake and glycogen deposition employing diaphragm sections obtained from a single rat

| Insulin sample | Response criterion | Dose ratio (S/U) | Relative potency of U Per cent | Range of potency (P = 0.95) Per cent | Limits of error (P = 0.95) Per cent |
|---|---|---|---|---|---|
| Insulin Toronto vs Insulin Toronto | G.U.* | 1:1 | 99 | 48-204 | 48-204 |
| | G.D.† | 1:1 | 106 | 81-156 | 77-147 |
| Insulin Toronto vs. NPH Insulin | G.U. | 1:1 | 104 | 72-139 | 69-156 |
| | G.D. | 1:1 | 113 | 92-164 | 82-169 |
| Insulin Toronto vs Insulin Toronto | G.U. | 1:5 | 92 | 45-221 | 41-204 |
| | G.D. | 1:5 | 98 | 64-147 | 65-151 |

S = standard U = unknown
P = probability
*G.U. = Glucose uptake
†G.D. = Glycogen deposition
Each value shown represents the weighted mean of five individual assays.

Five, two by two assays were performed under each of the three conditions and the combined results of each group of five assays are shown in table 5. Statistical evaluation of the assays was carried out according to Bliss and Marks.[2,3]

The relative potencies do not differ significantly from each other regardless of the response criterion used. Limits of error and the range of potencies were greater for glucose uptake than for glycogen deposition. The average Indices of Precision favor glycogen deposition as the preferred response ($\lambda = 0.30$ for glycogen, $\lambda = 0.51$ for glucose uptake).

The Chi-square test for homogeneity was applied in each case and potency ratios were homogeneous.

## DISCUSSION

The use of a single diaphragm for the complete assay of insulin has eliminated certain variables of the in vitro assay procedure and reduced the importance of other factors which have tended to decrease the reliability of many existing methods. Between-animals variation which has been demonstrated in this work has been completely eliminated by the use of one diaphragm for a complete assay. Other factors which have influenced the results in assaying insulin by in vitro technics, such as sex, age, weight and nutritional status of the animals no longer are of great importance. The internal controls existing when a single rat is used overcome many of these variables.

Comparison of the two methods of measuring insulin activity indicates that glycogen deposition has a lower Index of Precision ($s/b = 0.348$) than glucose uptake ($s/b = 0.574$). This is due to the lower standard error obtained in measuring glycogen synthesis. These Indices of Precision are obtained by analysis of the respective dose-response curves and they are higher than those determined for individual or combined assays.

Measurement of the glucose uptake as well as glycogen deposition is of twofold importance. It gives a second response as a check and it indicates, when no glucose is utilized, the presence of nonviable or inactive tissue. In assays using pooled tissues the viability of individual portions cannot be demonstrated.

An additional advantage found in the present assay method is the saving of time and animals which accrues. A result may be obtained with one rat which is equivalent to that obtained in other assays using eight to sixteen rats or forty mice. Further, four such results may be obtained in the same time taken with many other methods and without increasing equipment or personnel. The use of combined assays results in better estimates of the potency and narrower limits of error. It is quite feasible for a complete assay to be carried out by one individual.

## SUMMARY

A method has been described for the in vitro assay of insulin which measures the glycogen deposition in a single rat diaphragm. Concurrent estimations of the glucose uptake by the same tissues provide an additional response criterion as well as a check on tissue viability. A linear response to the log-dose of insulin was found over the range 25 to 10,000 *μ*U. Tissue size did not influence either glucose uptake or glycogen synthesis. This method completely eliminates between-animals variation and provides internal controls for such variables as age, sex and nutritional status.

## SUMMARIO IN INTERLINGUA

*Bio-essayage de Insulina con le Uso del Deposition de Glycogeno in un Sol Diaphragma de Ratto*

Es describite un technica de bio-essayage in vitro de insulina que mesura le deposition glycogenic in un sol diaphragma de ratto. Estimationes simultanee del acceptation de glucosa in le mesme tissu provide un additional criterio de responsa e, in plus, verification del viabilitate tissular. Esseva trovate linearitate del responsas (in representation logarithmic) a doses de insulina inter 25 e 10.000 μU. Le grandor del specimens de tissu exerce nulle influentia in le acceptation del glucosa e in le synthese del glycogeno. Iste technica elimina totalmente le variation inter differente animales e provide correctivas interne pro variabiles como etate, sexo, e le stato nutritional.

## ACKNOWLEDGMENT

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## *Vascular Disease and the Growth, Repair, Regeneration and Degeneration of Vascular Elastic Membranes*

Gillman and co-workers have been analyzing, first, the basic structure of vascular elastic membranes, their mode of growth and reactions to injury, and second, the intravascular factors which might play important roles in promoting or retarding mural thrombotic or fibrin deposits over sites of previous injuries and the effects on these of fat intake and sex hormones.

Their studies of the structure and postnatal development of the vascular elastic membranes, their reactions in human and experimentally induced lesions indicate that arterial elastic membranes are not homogenous in structure, i.e., they seem not to be comprised solely of "pure" elastic tissue, in contrast to what is the apparent case for the ligamentum nuchae. They suggest that the vascular elastic membranes are comprised of "collagen-like" hyaline "cores" enclosed by an elastic-like component, this whole complex, in turn, being sleeved by an irregular coat of mucopolysaccharide. This opinion is based upon their extensive morphological and histochemical studies of the vascular elastic membranes in man and rats at different ages and in various pathological states (*A.M.A. Arch. Path.* 59:733, 1955; 67:624, 1959; *Connective Tissue*, p. 128. Blackwell, Oxford, 1957; *J. Mt. Sinai Hosp.* 24:857, 1957).

Their recent analysis led these investigators to suggest that, in man, the vascular elastic membranes are remodeled during growth, an opinion to which they were initially led from studies of *Lathyrus*-intoxicated rats. Such remodeling may depend upon a continuous interchange of the molecular components of the various parts of the vascular elastic membranes, a process which may involve in some way the irregular perimembranous sleeves of mucopolysaccharide. They record that alterations in certain tinctorial reactions of elastic-like components of vascular elastic membranes, which occur spontaneously even in infants (perhaps as part of normal growth and remodeling) are almost invariably associated with increases in the mucopolysaccharide surrounding the areas of change (*A.M.A. Arch. Path.* 67:624, 1959).

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# Latent Diabetes

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Diabetes mellitus has been described as "chronic hyperglycemia . . . which may be either continuous or intermittent and which is due to a relative or absolute deficiency of insulin."[15] Joslin considers that "the tendency to diabetes is 'inborn' and must remain so for life."[9] "Permanency" is the universal concept of the diabetic state. Remission of diabetes following treatment has been reported[11] but in these patients the glucose tolerance tests remained diabetic in form, indicating that the clinical manifestations were controlled but that the underlying biochemical disturbance persisted. Disappearance of the diabetic state seldom occurs, and rigid criteria must be satisfied if a cure is to be claimed.[9] Isolated cases of "cure" or "remission" have been recorded[5,10,17,19,21] and one series of eight cases has been reported by Wells.[22] The occurrence of temporary diabetes in the tropics has recently received attention in the medical press.[1,15,21,23] As twelve patients in whom the manifestations of diabetes were of short duration have been encountered at the University College Hospital of the West Indies in the last six years, we feel that they should be reported. Two other diabetic patients who behaved in a similar manner have not been included in the series as no blood sugar estimation was made during the initial acute phase of their diabetes.

## MATERIAL

The clinical details of twelve diabetic patients treated at the University College Hospital of the West Indies are summarized in the table. These patients represent about 1.5 per cent of all the diabetic patients treated at the hospital since 1952. There were five males and seven females, their ages ranging from forty-four to fifty-four years and twenty-four to forty-three years respectively. Six were admitted to hospital in diabetic coma or precoma, four were admitted for stabilization of their diabetes and one was treated as an outpatient. One male with severe Weil's disease was found to have hyperglycemia and glycosuria on admission to the hospital. In all, the evidence of diabetes disappeared, but in five the disease has reappeared clinically. The interval between the initial hospitalization or outpatient treatment and the relapse was 33, 36, 43 and 52 months in four cases while one patient relapsed and recovered twice after thirty-two and thirty-eight months. Nine have put on more than ten pounds in weight and four of these have relapsed.

## DISCUSSION

Joslin states that the following conditions must be fulfilled for five years before cure of diabetes can be accepted: "Glycosuria and hyperglycemia must be absent while the patient is without diabetic medication, both before and one hour after a meal. The meal must contain two fifths of the carbohydrate for the day. The carbohydrate for the twenty-four hours should comprise at least two thirds of the calories necessary to provide thirty calories per kilogram body weight. Better still, the carbohydrate tolerance should be unimpaired as judged by a normal glycemic curve following the oral administration of 100 gm. of glucose in the postabsorptive state."[9] Only two of our patients (V.M. and I.Mc.) have so far been followed for five years. One (V.M.) still diets carefully and the second (I.Mc.) does not overeat. Both show very slight impairment of carbohydrate tolerance when tested with 50 gm. of glucose.

Remission of manifestation of diabetes is known to occur but is relatively uncommon. Weight reduction in the obese patient by adequate dietary restriction is commonly followed by amelioration of the diabetic state and a normal sugar tolerance may be regained.[11] Subsequent development of obesity is generally associated with the reappearance of the manifestations of diabetes. In this series, only three (R.T., O.F. and I.Mc.) were definitely obese at the onset of their disease. In none was there a sustained therapeutic weight reduction. Nine (D.L., R.T., D.E., O.F., J.S., S.H., V.M., V.R., K.H.) have put on appreciable amounts of weight and of these only four (D.L., D.E., V.R., K.H.) have relapsed.

Pregnancy may unmask a latent diabetic state.[8] It is of interest, therefore, that two of the patients in this series (O.F. and K.H.) became pregnant after regaining their normal sugar tolerance. In both, the pregnancy was un-

From the University College of the West Indies, Jamaica, W. I.

## TABLE 1

Clinical details of twelve patients with latent diabetes

| Name and number | Age (years) | Sex | Duration of symptoms prior to hospitalization | Reason for admission to hospital | Initial blood sugar mg. per 100 ml. | Total insulin in first twenty-four hours (units) | Duration of insulin therapy | Length of follow-up in months | Continued adherence to diet | Relapse | Most recent glucose tolerance test* | | | | | Weight when first seen (lbs.) | Present weight (lbs.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | F.B.S. | One-half hour | One hour | Two hour | Three hour | | |
| 1. F.C. | 34 | M | 2 weeks | Diabetic coma | 655 | 455 | 22 days | 36 | No | No | 74 | 125 | 105 | 66 | 57 | 159 | 156 |
| 2. D.L. | 38 | F | 5½ years | Diabetic coma | 1,200 | 1,220 | 37 days | 39 | Mainly | Yes after 33 months | 71 | 136 | 120 | 71 | 70 | 155½ | 180 |
| 3. R.T. | 41 | F | 3 months | Diabetic coma | 719 | 325 | 19 days | 15 | Mainly | No | 100 | — | 131 | 105 | 78 | 173½ | 194 |
| 4. D.E.† | 35 | F | 5 months | Diabetic precoma | 540 | 380 | 40 days | 57 | No | Yes after 52 months | 113 | 169 | 159 | 122 | 118 | 110 | 115 |
| 5. O.F. | 25 | F | 3 weeks | Diabetic precoma | 500 | 350 | 22 months | 46 | No | No | 88 | 108 | 126 | 109 | 102 | 186 | 210 |
| 6. J.S. | 47 | M | 4 weeks | Diabetic precoma | 1.050 | 405 | 34 days | 11 | Yes | No | 78 | 124 | 164 | 147 | 83 | 157 | 168 |
| 7. S.H. | 44 | M | Unknown | Weil's disease | 440 | 240 | 25 days | 26 | No | No | 89 | 105 | 152 | 131 | 89 | 106½ | 149½ |
| 8. V.M.‡ | 43 | F | 4 months | Stabilization | 350 | 60 | 38 days | 77 | Yes | No | 105 | 158 | 152 | 132 | 74 | 133½ | 145 |
| 9. I.Mc. | 47 | M | 1 week | Stabilization | 320 | 60 | 13 days | 63 | No | No | 84 | 132 | 156 | 102 | 70 | 208 | 209 |
| 10. V.B. | 54 | M | 6 weeks | Stabilization | 264 | 50 | 17 days | 48 | Irregularly | Yes after thirty-two and thirty-eight months | 84 | 142 | 147 | 89 | 74 | 114½ | 131 |
| 11. K.H. | 35 | F | 7 months | Stabilization | 264 | 20 | 6 months | 46 | No | Yes after forty-three months | 73 | 115 | 143 | 114 | 81 | 129 | 145 |
| 12. B.B. | 24 | F | 1 month | Treated as O.P. | 478 | 90 | 61 days | 48 | No | Yes after 36 months | 99 | 135 | 179 | 114 | — | 140½ | 145 |

*Glucose tolerance test: 50 gm. of glucose given by mouth except cases 2 and 3 who received 100 gm.
†This patient had put on 14 pounds in weight prior to relapse.
‡This patient was previously reported by Hugh Jones.[7]

eventful, no glycosuria developing. The babies weighed 7½ lb. and 9 lb. 3½ oz. One (O.F.) is now pregnant again forty-six months after the first appearance of diabetes and still has a normal blood sugar. However, the mother of the 9 lb. 3½ oz. baby (K.H.) has relapsed two years after the delivery. It may also be significant that only one of the twelve patients (I.Mc.) gave a family history of diabetes. This patient, although overweight, has been followed for sixty-three months. He does not adhere to a diet but does not overeat.

Infection was present in only one patient (S.H.) who was admitted with severe Weil's disease and found to have hyperglycemia and glycosuria prior to treatment with intravenous glucose. There was no preceding history of diabetes. Insulin administration controlled the diabetes and was stopped after twenty-five days. He has since remained well and no longer adheres to a diet. However, a glucose tolerance test modified by prior administration of 50 mg. of cortisone suggests that he is probably a potential diabetic. It is likely that the diabetes was related to the severity of the infection and not to the Weil's disease per se. No other patient with severe Weil's disease admitted to the University College Hospital of the West Indies has been found to have hyperglycemia and there is no evidence that the Leptospira invade the islets of Langerhans.

Mild diabetes may be revealed by a glucose tolerance test. In the series of cases herewith reported the upper limits of normal were as follows—50 gm. glucose tolerance: fasting level 100 mg. per 100 ml.; one-hour level 150 mg. per 100 ml. and two-hour level 100 mg. per 100 ml.; 100 gm. glucose tolerance: one-hour level 160 mg. per 100 ml. and two-hour level 110 mg. per 100 ml.

All estimations were done on venous blood by the method of Nelson as modified in the Pepper Laboratory, Hospital of the University of Pennsylvania. By these criteria, the two patients (D.L. and R.T.) who were tested with 100 gm. of glucose showed a normal response, as did two (F.C. and V.B.) tested with 50 gm. One of each pair (D.L. and V.B.) has relapsed, however. The remaining eight patients, tested with 50 gm. of glucose, all show minor deviations from the normal range, but only three (D.E., K.H. and B.B.) have relapsed clinically. In the other cases the diabetes is latent, being shown only by the minimal biochemical abnormality. Fajans and Conn[4] are investigating latent diabetes

by administering cortisone prior to carrying out a glucose tolerance test. A modification of their test was performed on two patients during their remission (D.E. and S.H.) and confirmed the fact that the minor abnormalities shown by the standard test were significant. One (D.E.) has relapsed after fifty-two months.

The fact that the twelve patients regained a normal or near normal tolerance for sugar would indicate that their pancreases were once again able to synthesize and secrete an adequate amount of insulin. That this should be so in the six patients admitted with diabetic coma or precoma is particularly interesting as Wrenshall et al.[21] have shown that patients dying from diabetic coma have virtually no extractable insulin in their pancreas. Whatever the cause of the acute islet failure or acute interference with insulin action in these patients, the condition was reversible, adequate function being restored in all, although two (D.L. and D.E.) who did not adhere carefully to their diet have relapsed after an interval of thirty-three and fifty-two months respectively. These six cases resembled the acute cases reported by Cheng et al.,[3] Harwood,[8] Phillips,[17] Peck et al.,[16] Wells,[22] Stutman and Hayes,[19] and Waddell.[21] It is also possible that they resemble the case reported by Taylor[12] who demonstrated the presence of both insulin and an insulin antagonist in the serum during the acute diabetic phase and no antagonist during the remission. Wrenshall[21] also showed that middle-aged diabetics had a reduced but variable insulin content of the pancreas. Adequate dietary restriction and weight reduction may accommodate the body to this diminished capacity of the islets to form and release insulin. In the five nonacute cases reported here, dietary modification may have played a part in their initial improvement, although only one was overweight. Only one patient (V.M.) still adheres carefully to a diet and after seventy-seven months is well, although showing minor biochemical abnormalities; one (V.B.) dieted irregularly and has relapsed; three stopped dieting and two (K.H. and B.B.) have relapsed, while the third (I.Mc.) shows a minimal biochemical impairment. Three of those who relapsed (D.L., V.B. and K.H.) had put on 16 lb. or more in weight prior to the reappearance of the diabetes. These facts would suggest that the basic defect responsible for the development of diabetes persists in these patients, and that in some, unrestricted diet and increase in body weight may have played a part in the redevelopment of a frank diabetic state.

MacLean and Ogilvie[20] have shown that in patients dying early in the course of diabetes the islets of Langerhans are hypertrophied. This finding corresponds to the clinical observation of Brush[2] that early aggressive treatment of diabetes in the young was likely to result in a remission of symptoms for some time. Adequate treatment at this phase of the disease may result in restoration of normal or near normal islet cell function. Five of the cases reported here were treated within one month of the onset of their symptoms and five between six weeks and seven months, but this situation does not apply to the patient who had been diabetic for five and one-half years prior to developing diabetic coma.

Whether or not conditions in the tropics predispose to the occurrence of temporary diabetes provides an interesting topic for speculation. In the tropics the daily intake of total calories is seldom excessive, frequently is less than generally accepted for well developed countries, and may fluctuate considerably from day to day. The intake of first class protein is low or negligible. In the experimental animal a low protein diet increases the sensitivity of the animal to alloxan,[6] while it has been pointed out by Lazarow[10] that "low protein diets would tend to accentuate deficiencies of cysteine and methionine and might, therefore, decrease the ability of the beta cells to synthesize insulin, and thus contribute to the diabetic state." In Jamaica, the diet of the hospital class of patients is from childhood generally deficient in first-class protein. Carbohydrates usually provide the bulk of the calories and most of this is consumed at one or two meals. Houssay and Martinez[9] have shown that the 95 per cent depancreatized experimental animal is readily made diabetic by administering a high carbohydrate diet (59 per cent of the total calories). It is therefore possible that after twenty to forty years, this tropical dietary pattern may lead to insufficiency of islet function even in the absence of an excessive daily intake of calories. Prompt and adequate treatment of the ensuing diabetic state can presumably rehabilitate the islet cell for a variable period during which the diabetic state becomes latent. This possibility deserves long-term investigation in the experimental animal.

SUMMARY

The records of twelve patients in whom the clinical and biochemical manifestations of diabetes mellitus were of limited duration are reported and discussed. Six presented in diabetic coma or precoma. All have been followed up for periods varying from eleven to seventy-seven months. Five have relapsed. Three who have not relapsed no longer adhere to a diabetic-type diet. All are considered to be in or to have been in a latent phase of their diabetes.

SUMMARIO IN INTERLINGUA

*Diabete Latente*

Es reportate e discutite le protocollos de dece-duo

patientes in qui le manifestationes clinic e biochimic de diabete mellite esseva de duration restringite. Sex se presentava in coma o precoma diabetic. Omnes ha essite sub observation subsequente durante periodos de inter dece-un e septanta-septe menses. Cinque ha suffrite recidivas. Tres—sin recidiva—ha cessate observar regulas diabetic de dieta. Omnes es reguardate como essente o como habente essite in un phase latente de diabete.

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## *Vascular Disease and the Growth, Repair, Regeneration and Degeneration of Vascular Elastic Membranes*

Gillman and co-workers suggest that, in the presence of vascular injuries of developmental origin, the already low plasma fibrinolytic activity (apparently hormonally determined in males) may perhaps be further depressed by regular high fat intakes, thus promoting (particularly in males) endarterial fibrin silting such as that described by J. B. Duguid (*Brit. Med. Bull. II*:36, 1955), especially at sites of previous arterial injuries (*Lancet II*:1117, 1957; *II*: 901, 1958). Malnutrition may decrease endarterial silting not only by its effect on blood lipids but also by associated endocrine changes (*Ciba Foundation Colloquia on Aging* 3:104, 188, 1957; *Lancet II*: 696, 1957).

These workers are thus of the opinion that the pathological changes within arteries will predispose to actual occlusive episodes only under appropriate metabolic conditions. They therefore favor a multiple factor etiology of the occlusive episodes in association with previously established coronary pathology of developmental origin. They consider that their own observations may provide an explanation for the different frequencies of coronary occlusive episodes in different races, despite the almost equal incidence of pathological changes in coronary and other arteries in different populations recently described by Holman and co-workers (*Am. J. Path. 34*:209, 1958).

*In summary*: These investigators believe that there is probably no single cause of degenerative arterial diseases or of terminal thrombosis. The final common path probably involves two independent but usually concurrent processes: (1) the vascular lesions resulting from deranged growth, remodelling, regeneration or repair of acute injuries to arterial walls themselves, and (2) the factors in the circulating blood (influenced by diet and hormone balance) which promote or suppress localized intimal fibrin deposits, apparently most often formed over rigid intramural vascular scars (*Excerpta Medica, Sect. XX, 2*:367, 1959).

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# Tolerance for Glucose in the Partially Depancreatized Dog During Prolonged Administration of Tolbutamide

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There is convincing evidence that a single injection of tolbutamide into a peripheral vein or the pancreaticoduodenal artery of the dog stimulates the release of insulin from the pancreas.[1] Whether or not this occurs in man under similar conditions, whether it occurs upon chronic oral administration in either man or animals, and whether, if it does occur, insulin production is stimulated in addition to insulin release, are still unanswered questions.

The possibility that insulin production may be increased has led many to fear that the already deficient beta cells of the diabetic patient might eventually become exhausted under long-term treatment with tolbutamide. Indeed, this has been proposed as one explanation of the so-called secondary failures in sulfonylurea therapy.

It seemed likely that some light might be shed on this problem by observing the effect on glucose tolerance of the prolonged administration of tolbutamide to animals whose beta cells had been reduced by subtotal pancreatectomy. This report describes such experiments.

## METHODS

Ten healthy, adult mongrel dogs weighing from 8.1 to 14.5 kg. and selected for their relative docility were partially depancreatized so as to leave a small remnant in the region of the main pancreatic ducts. The size of the remnant, estimated by fashioning and weighing a fragment of similar size from the extirpated portion, ranged from 12 to 32 per cent of the whole gland with a mean of 20 per cent.

Beginning generally three to four weeks after operation each dog was given a preliminary series of glucose tolerance tests at intervals of one to sixteen weeks over periods of from four to twenty months for a total of at least five tests. After a seventeen-hour fast, and with the animal lying on its side under a minimum of restraint, 1.0 ml. per kg. of a 50 per cent glucose solution was injected intravenously during three minutes. Venous blood samples were taken before and, from a separate site, 15, 30, 45, 60, 90 and 120 minutes after injection and analyzed for "true" glucose by the method of Nelson.[2]

After the preliminary series of tests the animals were divided into two groups of five each. The first group received no treatment. The second was given orally 30 to 40 mg. of tolbutamide per kg. per day in divided doses over periods of thirteen to seventeen months, with appropriate precautions to ensure actual swallowing of the drug. Glucose tolerance tests were continued at intervals in both groups, tolbutamide being withheld for five to seven days before all tests in the drug-treated group. Each dog received a total of nine to fourteen tests. All animals were housed in metabolism cages and weighed periodically. They were given one to two cans of Rival dog food daily to maintain relatively constant body weight. At the end of the experiment the dogs were sacrificed, the pancreatic remnants weighed, and samples of liver, kidney and pancreas processed for histologic examination. A comparable group of six normal dogs was given a series of three glucose tests by the method described.

## RESULTS

Comparison of the estimated weight of the pancreatic remnant at operation with its measured weight at autopsy showed an increase of 1.5 to almost fourfold with no significant difference between treated and untreated groups. No animal had steatorrhea or other evidence of pancreatic exocrine deficiency.

Four of the five dogs receiving tolbutamide lost weight (mean, 1.7 kg.) while all untreated dogs gained (mean, 3.1 kg.), despite efforts to minimize

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such changes by controlling food intake. Prothrombin time was unaltered in two dogs tested after nine months of tolbutamide therapy.

Histologic examination of liver and kidneys revealed no abnormalities or differences beyond those expected in mongrel pound dogs. Sections of pancreas were stained with hematoxylin-eosin, chromium hematoxylin, and aldehyde fuchsin. Islets were numerous in almost all the dogs but were smaller, less discrete and more irregular in the treated animals (figure 1). Occasionally in this group a few large islets were seen and these often showed unusual interdigitation with acinar tissue. One such large islet demonstrated transformation from an acinar lobule. Of the five treated dogs, four showed poor beta-cell granulation. All five untreated animals had numerous beta-cell granules (figure 2).

Glucose tolerance curves in the experimental dogs, whether or not treated with tolbutamide, were rarely outside the range of curves obtained in normal dogs. When this became apparent it was decided to attempt the difficult task of determining whether, within normal ranges, there was any significant difference in blood sugar behavior between tolbutamide treated and untreated animals. Since comparison of glucose tolerance curves as such is difficult, the following criteria, each expressed as a single figure, were employed:

1. The mean fasting blood sugar value.
2. The mean one-hour blood sugar value.
3. The mean rate of blood glucose disappearance as measured by the slope of the curve between fifteen and sixty minutes using the formula $2/7[-4x_1 - x_2 + 5x_3]$ in which $x_1$, $x_2$ and $x_3$ represent the blood sugar values at 15, 30 and 60 minutes, respectively. The formula describes the descending limb of the glucose tolerance curve as a negative number which increases in negativity with increasing steepness of slope.

In addition, the "diabetic index" proposed by Lazarow[3] was calculated but proved too insensitive for our conditions.

Mean fasting blood sugar values (table 1) were slightly above mean normal values in the two groups of operated dogs during the first five tests and prior to

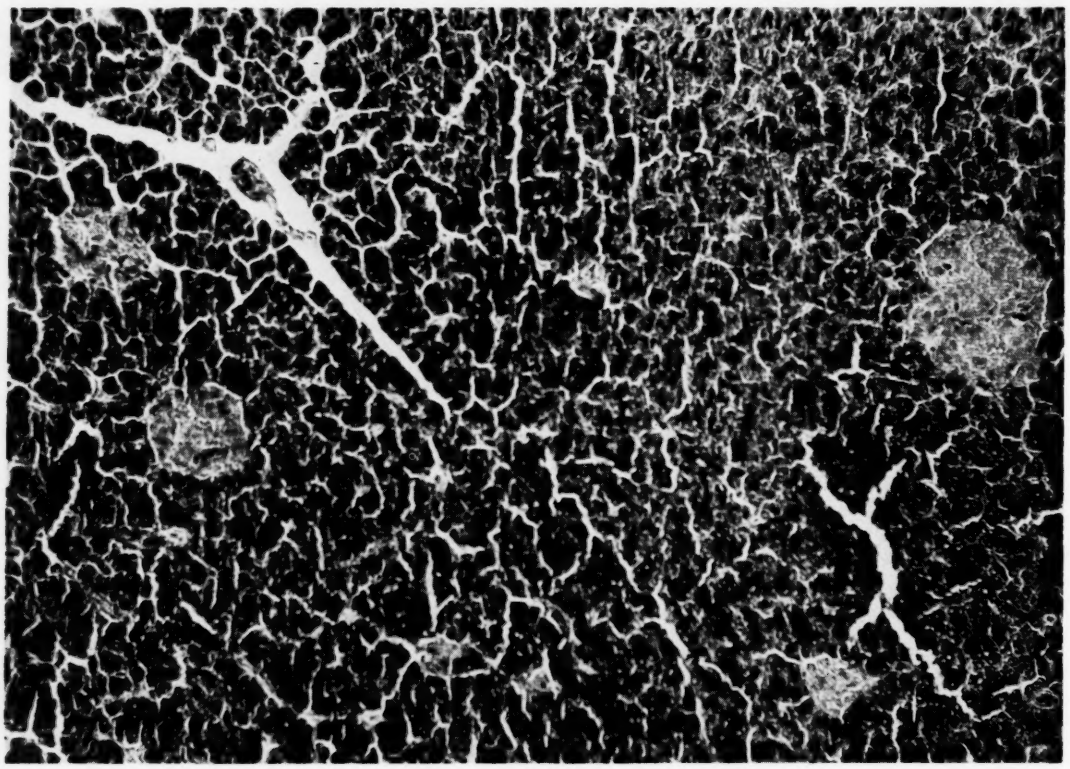

FIG. 1 (A). Pancreatic islets of a dog (11-P) which did not receive tolbutamide are normal in appearance and size. (All photomicrographs are of aldehyde fuchsin (Gomori) stained material.) Magnification 140 X.

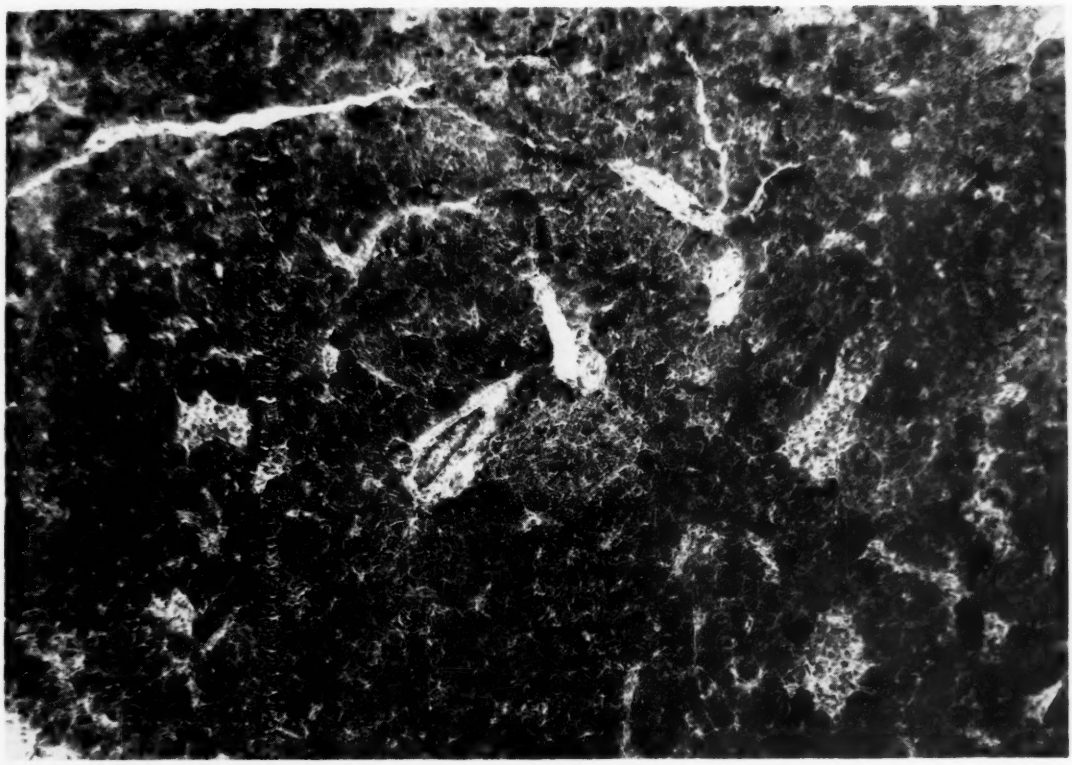

FIG. 1 (B). Islets of a dog (22-P) which received tolbutamide for a year tend to be smaller, more irregular and less discrete. The total number of islets is equal to or greater than that seen in untreated animals. The acinar tissue is overstained. Magnification 135 X.

TABLE 1

Mean fasting blood sugar in partially depancreatized dogs

| No tolbutamide | I (First 5 GTT's) | II (Last 5 GTT's) | |
|---|---|---|---|
| HA-11-P | 87 | 74 | |
| HA-12-P | 86 | 64 | |
| HA-13-P | 84 | 71 | |
| HA-25-P | 54 | 51 | |
| HA-26-P | 71 | 65 | |
| Mean of means | 76 | 65 | Mean diff. 11* |
| **Tolbutamide treated** | | | |
| | Before tolbutamide (5 to 8 GTT's) | During tolbutamide (4 to 6 GTT's) | |
| HA-14-P | 98 | 70 | |
| HA-15-P | 83 | 60 | |
| HA-21-P | 74 | 42 | |
| HA-22-P | 71 | 48 | |
| HA-23-P | 85 | 56 | |
| Mean of means | 82 | 55 | Mean diff. 27* |

Tolbutamide omitted for five days prior to each glucose tolerance test.

Normal controls
Six normal dogs (mean of thirty-six determinations) 67±11

*p = <.01

administration of tolbutamide. During the last five tests, while the treated group was receiving tolbutamide except for a few days before each test, fasting values declined in both treated and untreated groups, but the decline was significantly greater in the former ($p<0.01$).

Mean one-hour blood sugar values (table 2) in both experimental groups were higher than mean normal values in the first five tests (prior to tolbutamide). In the last five (tolbutamide period) they declined toward normal in both, but the decline in the treated group was less (10 mg./100 ml.) than in the untreated group (18 mg./100 ml.). This is, however, not statistically significant ($p=0.3$).

The mean rate of glucose disappearance (table 3) in later as compared with earlier tests showed an increase in steepness of slope (hence increased "tolerance") in the untreated group (—22) while the treated group showed a decrease (hence decreased "tolerance") (+14). The algebraic difference between these changes (36) is statistically significant ($p<0.05$).

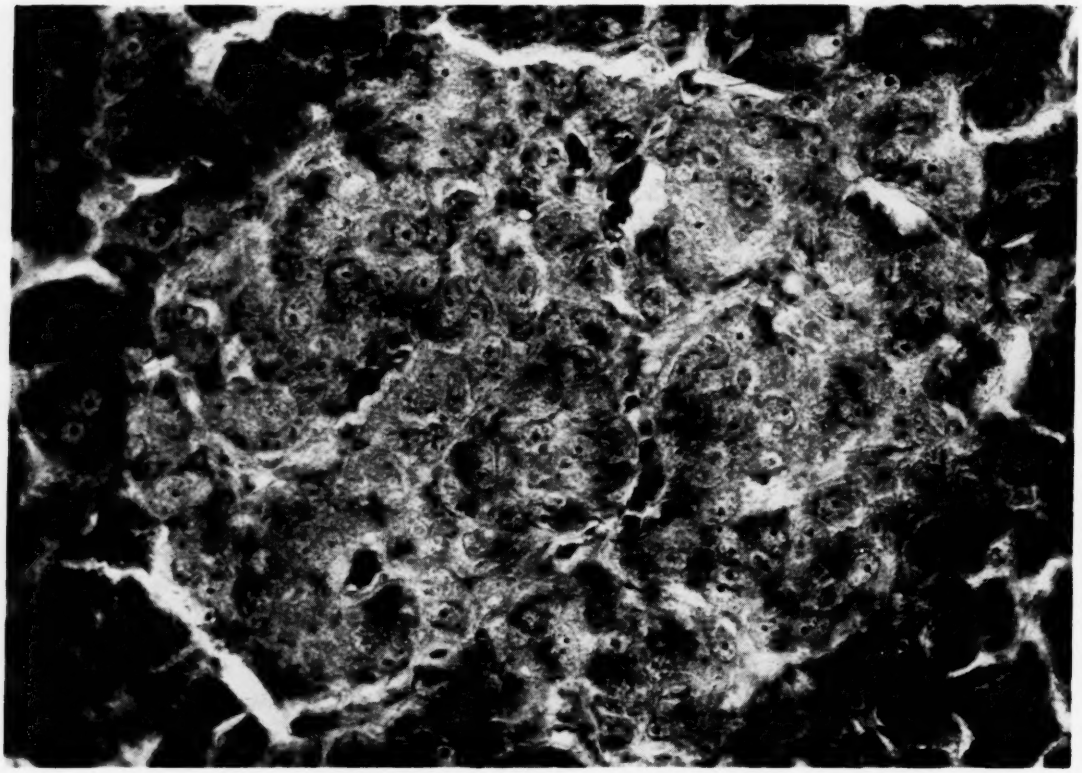

FIG. 2 (A). Beta-cell granulation (dark gray areas) in the islet of a dog not given drug appears normal as does the configuration of the islet itself. Magnification 725 X.

TABLE 2

Mean blood sugar one hour after intravenous glucose 0.5 gm./kg. in partially depancreatized dogs

| No tolbutamide | I (First 5 GTT's) | II (Last 5 GTT's) | |
|---|---|---|---|
| HA-11-P | 108 | 74 | |
| HA-12-P | 100 | 70 | |
| HA-13-P | 102 | 88 | |
| HA-25-P | 81 | 83 | |
| HA-26-P | 87 | 72 | |
| Mean of means | 95 | 77 | Mean diff. 18* |
| Tolbutamide treated | Before tolbutamide (5 to 8 GTT's) | During tolbutamide (4 to 6 GTT's) | |
| HA-14-P | 107 | 93 | |
| HA-15-P | 112 | 95 | |
| HA-21-P | 85 | 89 | |
| HA-22-P | 84 | 81 | |
| HA-23-P | 120 | 100 | |
| Mean of means | 102 | 92 | Mean diff. 10* |

Tolbutamide omitted for five days prior to each glucose tolerance test.

Normal controls

Six normal dogs (mean of eighteen determinations) 71 ± 13

*p = .3

TABLE 3

Mean slope in partially depancreatized dogs

| No tolbutamide | I (First 5 GTT's) | II (Last 5 GTT's) | |
|---|---|---|---|
| HA-11-P | —87 | —136 | |
| HA-12-P | —77 | —103 | |
| HA-13-P | —77 | —109 | |
| HA-25-P | —96 | — 80 | |
| HA-26-P | —76 | — 97 | |
| Mean of means | —83 | —105 | Mean diff. — 22* |
| Tolbutamide treated | Before tolbutamide (5 to 8 GTT's) | During tolbutamide (4 to 6 GTT's) | |
| HA-14-P | — 95 | —116 | |
| HA-15-P | — 81 | — 68 | |
| HA-21-P | —102 | — 63 | |
| HA-22-P | — 90 | — 61 | |
| HA-23-P | — 56 | — 48 | |
| Mean of means | — 85 | — 71 | Mean diff. + 14* |

Tolbutamide omitted for five days prior to each glucose tolerance test.

Normal controls

Six normal dogs (mean of eighteen determinations) —81±26

*p = <.05

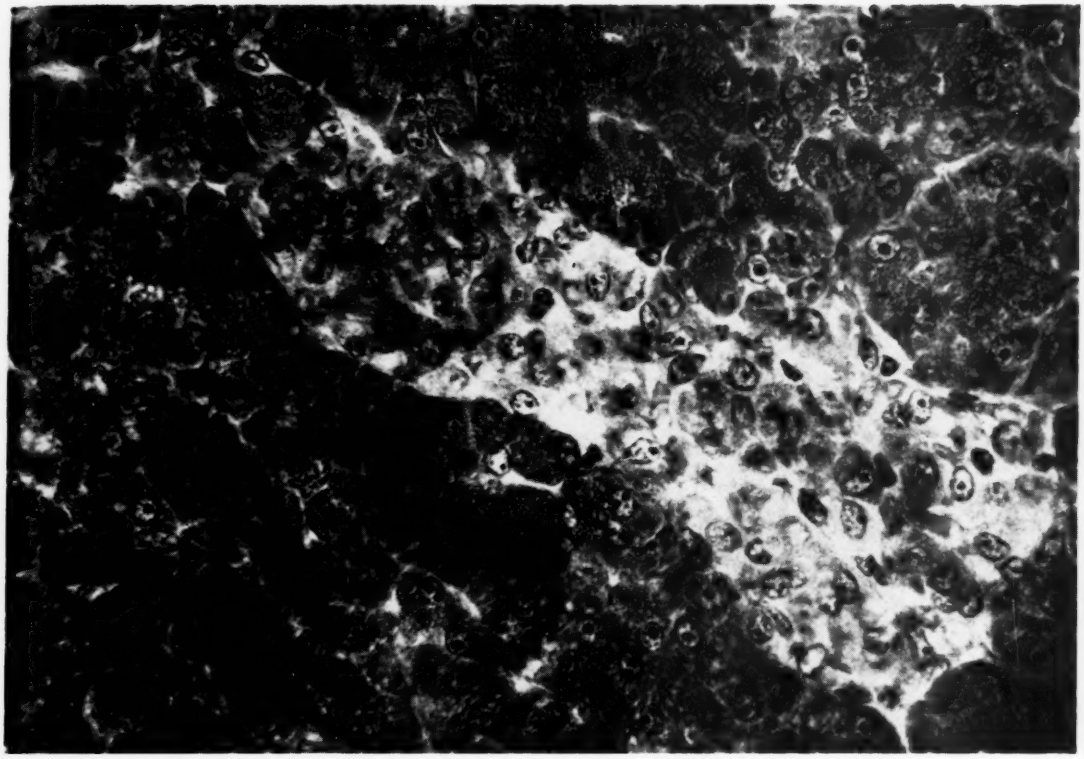

FIG. 2 (B). Diminished beta-cell granulation is seen in an islet of a treated dog. Interdigitation with acinar tissue is evident. Magnification 725 X.

## DISCUSSION

The extent of pancreatic regeneration in subtotally depancreatized dogs was somewhat surprising. Clearly, it was not influenced by administration of tolbutamide.

The mild loss of weight (1.7 kg.) among treated dogs compared with distinct gains (3.1 kg.) in untreated dogs is probably explained by an anorectic action of the drug. The severely toxic effects seen frequently in totally depancreatized dogs, however, were not observed in the present experiments, and histologic examination of kidney and liver revealed no abnormalities attributable to tolbutamide. Disturbances of size and form of islets among treated animals were real but of uncertain import. The observed diminution in beta-cell granulation is subject to the usual alternative explanations of degenerative change, increased release of insulin, or decreased production. The data here reported do not permit a clear decision between these possibilities. However, the failure of any animal to become diabetic suggests not only that the depletion of islet tissue by subtotal pancreatectomy was not severe in either group but also that prolonged administration of tolbutamide fell short of exhausting the remaining beta cells. What would have happened without partial regeneration of the pancreas is of course open to question.

Treated animals demonstrated a significantly slower rate of glucose disappearance from the blood as measured by determinations of slope. Similarly, though not as significant statistically, the mean one-hour blood sugar values showed a smaller decline between the first and last series of tests in the treated group than in those untreated. These findings suggest some decrease in glucose tolerance. The small magnitude of this decrease, however, occurring within the normal range, is of doubtful biological significance and is less than the impairment of tolerance found by Lazarow in alloxanized "subdiabetic" rats treated for months with tolbutamide. The difference may be accounted for by Lazarow's use of (a) rats instead of dogs, (b) alloxan, by which all the alpha cells are preserved, (c) animals that were more nearly diabetic in the beginning, (d) larger doses of tolbutamide (200-500 mg./kg. compared with our

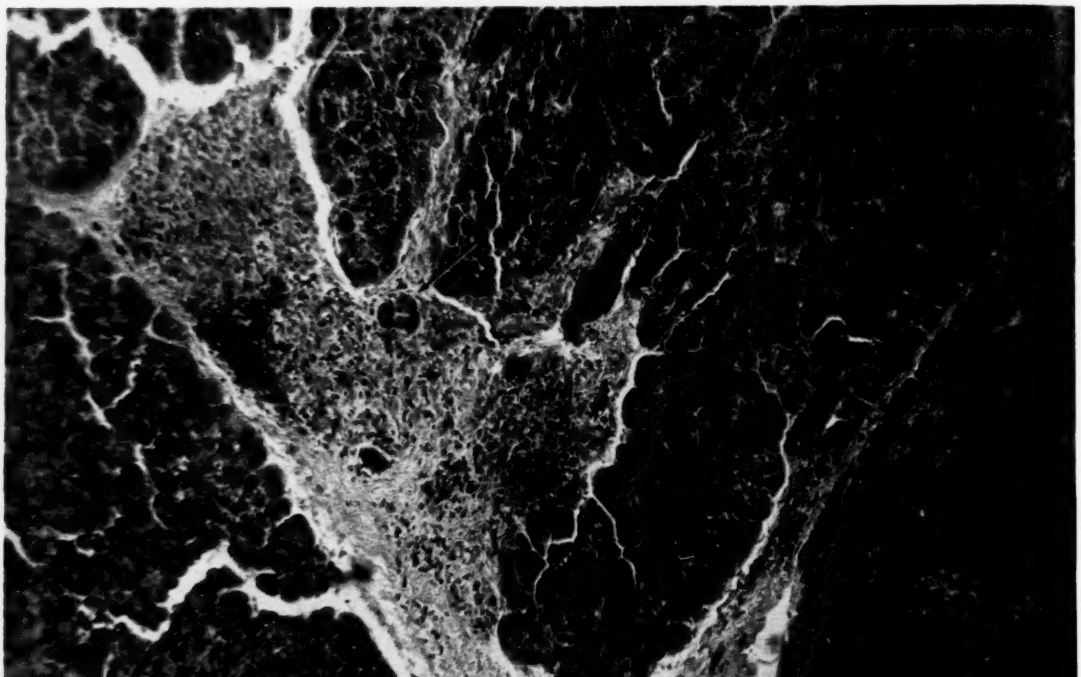

FIG. 2 (C). An unusual, large islet from a treated dog shows marked interdigitation with acinar tissue and transformation of an acinar lobule to islet tissue (arrow). Magnification 145 X.

30-40 mg./kg.). In addition, glucose tolerance tests in Lazarow's experiments were performed sixteen hours after the last dose of tolbutamide compared with an interval of five to seven days in the present experiments.

The greater decline, with time, in the fasting blood sugar of treated animals as compared with controls is in contrast to the absence of increase in tolerance for glucose seen in the former. This apparent paradox suggests that tolbutamide has induced an alteration in the function of pancreatic islets. If the beta-cell granules represent stored insulin, and if tolbutamide acts to release insulin from these stores, then long-term administration of the drug would be expected to produce two of the phenomena here described—a moderate decrease in beta-cell granulation and a lowering of values for fasting blood sugar. This same depletion of stored insulin, while not severe enough actually to impair tolerance for glucose, might be sufficient to prevent an increase in tolerance comparable to the decline in fasting blood sugar levels. It will be recalled that tolbutamide administered to responsive diabetic patients exerts a greater effect on the blood sugar during fasting than after ingestion of glucose.

## SUMMARY

Tolbutamide was administered to five subtotally depancreatized dogs over periods of thirteen to seventeen months. Another group of five such animals, not treated with tolbutamide, served as controls. A 1.5 to fourfold regeneration of pancreatic tissue occurred in both groups and was uninfluenced by the drug. The pancreatic islets of the treated dogs were smaller, more diffuse and irregular, and showed distinctly poorer beta-cell granulation than those of untreated dogs. No animal, however, became diabetic. As compared with preliminary glucose tolerance tests carried out in both groups, tests performed later showed in treated animals higher mean one-hour blood sugar values (not statistically significant) and slower mean rates of blood glucose disappearance ($p<.05$) but, curiously, lower mean fasting blood sugar levels ($p<.01$) than in untreated controls. These changes in blood sugar behavior among

the experimental group, taken together with the diminution in beta-cell granulation, are consistent with the hypothesis that prolonged administration of tolbutamide stimulated release of insulin from its storage form within the beta cell, thus lowering the fasting blood sugar but at the same time reducing the intracellular insulin reserves so as to prevent a corresponding increase in ability to dispose of a glucose load.

SUMMARIO IN INTERLINGUA

*Tolerantia pro Glucosa in Partialmente Dispancreatisate Canes Durante un Perdurative Administration de Tolbutamida*

Tolbutamida esseva administrate a cinque subtotalmente dispancreatisate canes durante periodos de inter dece-tres e dece-septe menses. Un altere gruppo de cinque similemente operate sed non tolbutamidisate animales serviva como controlo. Un regeneration de grados inter sesquiplice e quadruplice occurreva in le tissu pancreatic del animales in ambe gruppos e non esseva influentiate per le droga. Le insulas pancreatic del tractate canes esseva plus micre, plus diffuse, e irregular e monstrava un granulation de cellulas beta distinctemente inferior a illo notate in le canes non tractate. Tamen, nulle del animales deveniva diabetic. In comparation con tests preliminari del tolerantia pro glucosa que habeva essite effectuate in ambe gruppos, tests de data subsequente revelava in tolbutamidisate animales plus alte valores medie pro le sucro sanguinee post un hora que in le animales de controlo (non statisticamente significative) e un plus lente disparition del glucosa sanguinee ($p<0{,}05$), durante que—curiosemente—le nivellos medie de sucro sanguinee in stato jejun esseva plus basse ($p<0{,}01$). Iste alterationes in le comportamento del sucro sanguinee in le gruppo experimental—insimul con le diminution del granulation de cellulas beta—es congrue con le hypothese que le prolongate administration de tolbutamida es un stimulante del liberation de insulina ab su forma magasinate intra le cellulas beta, con le resultato que le sucro de sanguine in stato jejun es reducite e que simultaneemente le reservas de insulina intracellular es diminuite de maniera que un augmento correspondente in le capacitate de disponer de un carga de glucosa es prevenite.

ACKNOWLEDGMENT

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We gratefully acknowledge the assistance of Mr. K. Alexander Brownlee, Department of Statistics, The University of Chicago, in the development of the formula for rate of blood glucose disappearance and in statistical evaluation of the data. We are indebted to Dr. Eleanor Humphreys, Department of Pathology, for her careful review of all histologic preparations.

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Evidence has been presented consistent with the hypothesis that chronic electrical stimulation delivered to certain hypothalamic areas significantly intensifies both the rate of increase and the amount of hyperlipemia in rabbits fed a lipid-enriched diet. Such animals also showed a much greater atherosclerotic involvement of their aortic and coronary vasculature than did control animals given a similar diet. Those rabbits receiving stimulation in or near the ventral medial nucleus appeared to exhibit no greater hyperlipemia but considerably more atherosclerosis than the animals stimulated elsewhere.

It is concluded that central nervous system mechanisms exist which, under certain conditions, are capable of significantly influencing arterial atherogenesis.

From EFFECT OF CHRONIC HYPOTHALAMIC STIMULATION UPON CHOLESTEROL-INDUCED ATHEROSCLOROSIS IN THE RABBIT, by C. G. Gunn, Meyer Friedman and Sanford O. Byers, in *The Journal of Clinical Investigation*, Vol. 39, pp. 1963-72, Dec. 1960.

# Evaluation of Sorbitol in the Diet of Diabetic Children at Camp

*Jurgen Steinke, M.D., Francis C. Wood, Jr., M.D., Luis Domenge, M.D., Alexander Marble, M.D., and Albert E. Renold, M.D., Boston*

The practical impossibility of achieving perfect control with diet, insulin and other available therapeutic procedures in all but a few instances of juvenile diabetes is widely recognized. If defective glucose utilization is considered to be a likely primary lesion in diabetes, then it is evident that it might be useful to supplement therapy with insulin by the administration of substances metabolically similar to glucose, yet capable of being metabolized by the diabetic as well as by the normal. Indeed, in the pre-insulin era this idea, based on clinical observations of Külz in 1874,[1] and Bouchardat in 1883,[2] further stimulated by Minkowski's observation of adequate synthesis of liver glycogen from fructose in the pancreatectomized dog[3] provided what was perhaps the most hopeful approach to the treatment of the disease by means other than progressive starvation. The evidence that the severely diabetic human organism can oxidize fructose was, and remains, striking.[4,5]

The present study is concerned with the re-evaluation of the metabolism and possible usefulness in normal and in diabetic individuals of substances closely related to glucose, in this instance sorbitol. Before outlining the reasons for selecting sorbitol, the metabolism of fructose and sorbitol will be briefly reviewed. Emphasis will be given to more recent observations, since early studies with sorbitol have been reviewed by Olmsted[6] in 1953, just prior to the latest revision of biochemical knowledge concerning fructose and sorbitol metabolism.

## METABOLISM OF SORBITOL AND FRUCTOSE

As shown in figure 1, sorbitol is the polyalcohol resulting from the reduction of either the aldohexose, glucose, or the ketohexose, fructose. In mammalian metabolism sorbitol arises from the enzymatic reduction of glucose in the presence of reduced triphosphopyridine nucleotide (TPNH), a reaction which occurs in the placenta and in such accessory male sexual tissues as the seminal vesicle or prostate.[7,8] Once present in the mammalian organism, the metabolism of sorbitol requires initial oxidation to fructose, with simultaneous reduction of diphosphopyridine nucleotide (DPN). This reaction, catalyzed by the enzyme sorbitol dehydrogenase,[8,9] occurs primarily in hepatic and also in renal[10] tissue. In man, sorbitol is rapidly metabolized when administered intravenously in large amounts,[11] suggesting that sorbitol dehydrogenase is present in abundance. Subsequent to this oxidation, the metabolism of sorbitol is that of fructose, which has been extensively reviewed,[8,12,13] and which is outlined in figure 2, modified from Leuthardt's recent scheme.[12]

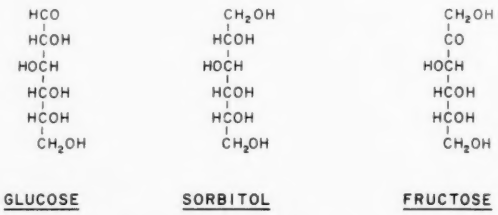


FIG. 1. Formulae of glucose, sorbitol and fructose.

In this scheme the heavy arrows indicate pathways of hepatic fructose metabolism which are of major quantitative importance. In liver and kidney fructose phosphorylation is primarily catalyzed by a separate kinase, fructokinase, and the further metabolism of the resulting fructose-1-phosphate merges with glycolysis at two distinct sites: (a) at the level of dihydroxyacetone phosphate, and (b) at the level of glyceric acid-2-phosphate. In the latter instance glyceraldehyde is oxidized to glyceric acid with simultaneous reduction of DPN[12,14,15] and glyceric acid is subsequently phosphorylated to glyceric acid-2-phosphate.[12,16,17] Glyceraldehyde may also be reduced in the presence of DPNH to glycerol,[12,18] which is then phosphorylated to a glycerol phosphate. Whether the direct phosphorylation of glyceraldehyde to phosphoglyceraldehyde[8] is a quantitatively important pathway is as yet uncertain; this is still Hers' favored hypothesis (personal communication). The metabolism of fructose, or of sorbitol, by the liver thus leads to the occurrence in hepatic tissue of at least three substances which are not usual products of the intermediary metabolism of glucose, namely fructose-1-phosphate, and free (nonphosphorylated) glyceraldehyde and glyceric acid. These intermediates, however, can rap-

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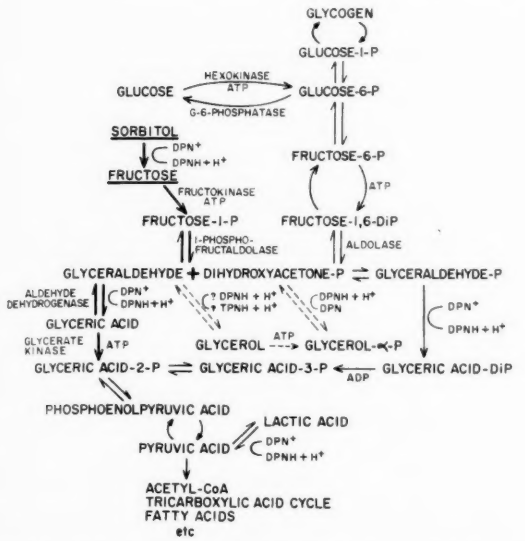


FIG. 2. Major pathways of sorbitol and fructose metabolism in liver. Not apparent on this schema are the following pathways: (a) direct phosphorylation of fructose to fructose-6-phosphate in the presence of hexokinase, a relatively minor pathway of fructose metabolism in liver; (b) phosphorylation of fructose-1-phosphate to fructose-1,6-phosphate, a reaction which may occur in muscle;[12] (c) phosphorylation of glyceraldehyde to phosphoglyceraldehyde in the presence of the enzyme triokinase, a possibility still favored by Hers.[5]

idly enter the normal pathways of glycolysis, without evidence of significant accumulation. Fructose metabolism completely bypasses the early, insulin-dependent, stages of glucose metabolism in the liver.

The pathways of fructose and sorbitol metabolism which have been outlined in figure 2 apply almost exclusively to hepatic and renal tissue. Other tissues, such as muscle,[19,20] adipose tissue,[21] and brain,[22] probably utilize fructose only by initial phosphorylation to fructose-6-phosphate, a reaction catalyzed by the enzyme hexokinase, which has a greater affinity for glucose than for fructose. Accordingly, in the presence of circulating glucose, fructose is metabolized to only a small degree by tissues other than liver and kidney.[23] In the presence of inadequate glucose phosphorylation by extrahepatic and extrarenal tissues (as in diabetes mellitus) it is evident that similarly inadequate fructose phosphorylation by these tissues is likely to prevail.

*Rationale for the use of fructose or sorbitol in diabetes.* The rationale for the use of fructose or sorbitol in the therapy of diabetes mellitus derives from acceptance of the concept that glucose utilization in general, and glucose phosphorylation by the liver in particular[24,25] is decreased in diabetes mellitus and, on the other hand, on the assumption that fructose phosphorylation and sorbitol dehydrogenation by diabetic liver proceed at a normal rate. Normal fructose phosphorylation by tissue from diabetic subjects has been observed directly in isolated liver preparations of experimental animals[26,27] and this is well supported in man by the observation that patients with severe diabetes mellitus have a near-normal tolerance for intravenously administered fructose[5,11,28,29] and sorbitol.[11] As a result intermediates of carbohydrate metabolism should be more abundant in the liver of the fructose-treated than in that of the glucose-treated diabetic organism. In addition, accelerated accumulation of hepatic intermediates of carbohydrate metabolism in the fructose-treated diabetic organism leads to the equally well documented improved glycogen synthesis[3,21,27] and to increased outpouring of substances such as pyruvate and lactate (possibly also glyceraldehyde and glyceric acid) which then become available to peripheral tissues, even in the absence of insulin.[5,28,29,30] Also, the increased availability to the liver of a diabetic of intermediates of carbohydrate metabolism is likely to result in return toward normal of a number of metabolic anomalies which are secondary consequences of the abnormal utilization of glucose, such as ketogenesis and the synthesis of fatty acids or cholesterol, and possibly of proteins such as albumin or lipoprotein fractions. In animals with experimental diabetes, at least partial repair of some of these hepatic functions has indeed followed the administration of fructose.[31-34] In man, the observations of Joslin and his collaborators[4] early established that even in cachectic patients with severe juvenile diabetes, fructose utilization and metabolism are sufficient to effect a definite change in the over-all respiratory quotient whereas a similar amount of glucose is totally ineffective. Near-normal oxidation of sorbitol labeled with C-14 has been recorded in diabetic patients.[35]

These and similar observations have led to the use of fructose in clinical nutrition to a larger extent in Europe than in the United States, as indicated by reviews such as those by Stuhlfauth.[36,37] Lack of enthusiasm for this therapy in this country probably derives in part from the opinion that a large portion of fructose administered to patients with severe diabetes is metabolized to glucose, either during its intestinal absorption or as a result of its hepatic metabolism. The two major recent studies of possible application of fructose to diabetes therapy carried out in the United States have expessed this concern[5,30] and data from this laboratory, both on isolated liver[27] and from patients with severe diabetes[11] support it. However, in all of these instances, relatively large amounts of fructose were administered intravenously or by mouth. Many of the European studies[36,38,39] were based on the oral administration of *small* daily quantities of fructose. The extent of fructose metab-

olism to glucose by diabetic liver is very likely dependent upon the rate at which fructose is made available to the hepatic cell.

*Differences between sorbitol and fructose.* From the point of view of its hepatic metabolism (figure 2) sorbitol is the equivalent of fructose with the additional production, on a molecular basis, of one more molecule of DPNH. Studies with the intravenous infusion of sorbitol at a rate exceeding by far the maximal possible rate of its intestinal absorption have established that the initial dehydrogenation of the molecule is unlikely ever to be rate-limiting in man. The additional availability of DPNH is probably responsible for the greater "antiketogenic" effect of sorbitol first demonstrated in vitro by Blakely[9] recently confirmed in this laboratory (figure 3), as well as for its greater accelerating effect upon hepatic fatty acid synthesis from acetate.[40]

EFFECT OF FRUCTOSE AND OF SORBITOL ON KETOGENESIS BY LIVER SLICES FROM RATS FASTED FORTY-EIGHT HOURS

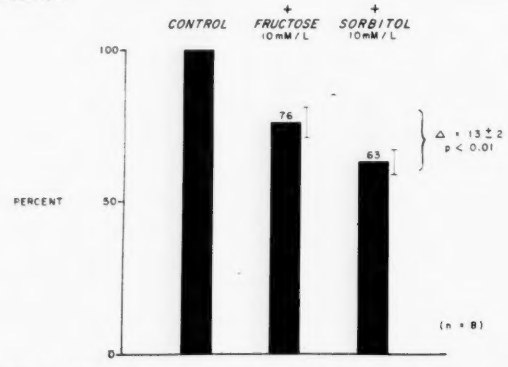


FIG. 3. Effect of fructose and of sorbitol on ketogenesis by liver slices from rats fasted forty-eight hours. Unpublished observations of Zahnd, Felber and Renold. Conditions and methods as described by Boshell et al.[45]

In addition, sorbitol differs from fructose quite markedly because of its extracellular distribution outside of liver and kidney, and because of differences in the intestinal and renal handling of fructose and sorbitol. Some of these differences have been summarized in table 1.

Although neither fructose nor sorbitol is actively absorbed by the intestinal mucosa, sorbitol is absorbed more slowly than fructose. The resulting delayed intestinal absorption explains its greater tendency to cause diarrhea when administered in large single doses, the greatest practical disadvantage of sorbitol over fructose. On the other hand, the same delayed intestinal absorption may make it possible to administer small quantities of available sugar to the diabetic organism over the

TABLE 1

Some biologic properties of sorbitol differing from those of fructose

1. Exclusive extracellular distribution outside of liver and kidney.[49]
2. Slower intestinal absorption, by diffusion only.[48,50]
3. Slow (or absent) metabolism by intestinal mucosa (Felber and Renold, unpublished observations, documented in table 2).
4. Ineffective (or absent) renal tubular reabsorption.[51]
5. Greater antiketogenic activity in liver slices (reference 9 and this report).
6. Greater accelerating effect on hepatic fatty acid synthesis from acetate.[40]
7. Marked cholekinetic effect.[52]
8. Possible effect on gastrointestinal absorption of certain vitamins.[53,54]
9. Less sweet than fructose.

greater portion of each day, and thereby tend to avoid the greatest potential disadvantage of fructose administration in diabetes, i.e., its excessive hepatic transformation into glucose. Sorbitol administration by mouth should result in a slow rate of availability of fructose to hepatic metabolism. Furthermore, sorbitol is either not metabolized by intestinal mucosa at all, or metabolized to a much smaller extent than fructose, as shown both with preparations of rat intestine (table 2) and in preliminary experiments with human intestinal mucosa, obtained by intubation biopsy. This may be of importance since the extent to which fructose is transformed into glucose during the process of intestinal absorption is an unknown quantity in man, and since it has been reported that a greater percentage of admin-

TABLE 2

Comparative utilization of fructose and sorbitol by rat intestinal wall (jejunum). Paired experiments in which 1 cm. lengths of jejunum were cleaned and split longitudinally into two halves. Incubation carried out in Krebs bicarbonate buffer. Unpublished observations of Felber and Renold

| Exp. No. | Fructose* | Sorbitol* |
|---|---|---|
| 1 | 6.7 | —0.5 |
| 2 | 2.5 | —0.3 |
| 3 | 2.1 | —0.2 |
| 4 | 2.5 | 0.0 |
| 5 | 2.4 | —0.4 |
| 6 | 4.2 | —0.4 |
| 7 | 4.1 | —0.2 |
| Mean | 3.5 | —0.3† |

* Expressed as milligrams fructose or sorbitol disappearing from the medium per 90 minutes and per gram wet tissue. Substrate concentration 10 mM/liter. Sorbitol measured according to West and Rapoport.[55]

† The small net increase in sorbitol during incubation is probably due to the release of small quantities of formaldehydogenic material during the incubation.

istered fructose is lost as glucose in urine as a result of oral rather than intravenous administration.[6] In experimental animals the extent of this transformation ranges from about 10 per cent in rats to 70 per cent in guinea pigs.[41]

In tissues other than the liver and kidney, sorbitol maintains an exclusively extracellular distribution. It is excluded from red blood cell water and, when given intravenously, high plasma levels result. This together with poor renal tubular reabsorption leads to considerable urinary loss of intravenously administered sorbitol.[13] However, this is of small importance when sorbitol is given by mouth, since its rate of absorption is so slow as to ensure almost complete removal during the first hepatic circulation. That urinary excretion of sorbitol does not exceed 2 per cent of the administered dose under the conditions described in this report has been checked in this laboratory.

Finally, sorbitol can be obtained at considerably less cost than fructose. Weight for weight, its sweetness is less marked, being approximately 60 per cent that of glucose; it resists both moist and dry heat in cooking.

## METHODS

Summer camps for diabetic children afford an unusual opportunity to observe a significant number of juvenile diabetics for periods varying between two and nine weeks. During summer camp (Elliott P. Joslin Camp, for boys, Charlton, Massachusetts), the daily program includes regular physical activities such as swimming, rowing, baseball, tennis and hiking, approximating home conditions much better than any hospital stay. Bed rest or restricted activities occur only as a result of intercurrent illnesses. Despite this free and relatively physiological environment, however, the physical seclusion of camp conditions and the availability of a camp laboratory and a camp physician and nursing staff, allow for an unusual degree of insight into, and control over, the diabetic state of the campers. Campers are on individually calculated, weighed and recorded diets, designed to provide adequate but not excessive food intake. Hypoglycemic reactions, injuries, infections, abnormal psychological reactions, and body weight at intervals are recorded on each camper's history sheet.

Since the age range is limited, usually between five and fifteen years, since duration and severity of diabetes are reasonably well known in each instance, and since daily activity patterns are relatively uniform, control groups for any planned study are relatively easily arranged for. Such control groups are especially important in order to account for the variation in outdoor activity from day to day which may be occasioned by changes in weather, and by activities such as hiking expeditions.

*Metabolic indices.* The following indices of metabolic control of the diabetic state were recorded and analyzed:

1. *The total daily dose of insulin.* Only the total dose per day was used in this analysis of data.

2. *The twenty-four-hour excretion of glucose in the urine.* Although this index ideally provides an excellent estimate of over-all carbohydrate balance, it loses much of its effectiveness as a result of the difficulties encountered in obtaining total twenty-four-hour collections. With camp children, deliberate discarding of the urine specimens or addition of tap water to urine bottles for concealment, are frequent and unpredictable means of normal juvenile mischief or disregard of rules. The urine collections were carried out using toluene as a preservative and the glucose content was estimated according to Sumner.[42] Creatinine determinations were not carried out and, accordingly, only unusually low total volumes suggested incomplete collections. The twenty-four-hour urine was tested for the presence of acetoacetate.

3. *Fasting blood samples* were obtained at intervals, as outlined below, and analyzed for *glucose, free fatty acids,* and *ketone bodies.* Sample bottles containing the appropriate amount of dry balanced oxalate were used for subsequent free fatty acid analysis, while sample bottles containing both balanced oxalate and fluoride were used for subsequent analysis for glucose and ketone bodies. The blood samples were immediately frozen. The determinations were carried out in the research laboratory in Boston. Glucose was measured by Nelson's method[43] after protein precipitation as suggested by Somogyi, free fatty acids by the method of Gordon[44] and ketone bodies according to the modification of the Greenberg and Lester technic described by Boshell and his collaborators.[45] To facilitate comparison with results from other laboratories, the values reported for free fatty acids are expressed in milliequivalents per liter of plasma, although the determinations were carried out on whole blood in order to permit rapid freezing at the camp. The titration figure obtained on whole blood hemolysates was corrected for the titratable material which exists in red blood cells ($0.47 \pm 0.08$ mEq./L.) which does not appear to be involved in lipid transport. Previous studies from this laboratory have shown that the concentration of titratable material of red blood cells is relatively constant in the presence of widely different plasma free fatty acid concentrations, both when values are compared in the same individual and when they are compared between individuals.[46] Also titratable material of red blood cells is not affected by the presence of diabetes or by insulin administration.

Fasting blood glucose determination was selected as perhaps the best established blood index of diabetes control. Free fatty acids were selected as the index of fat transport likely to change most acutely and, furthermore, as an index with as yet unknown potential warranting further definition. Blood ketone bodies were thought to represent a less labile index of compensation of lipid metabolism.

*Experimental design.* It was considered essential to arrange both for a parallel control group (i.e., campers present during the same period and following the same diet and program of physical activities, but not subjected to the variable under study) and for sequential controls within the groups themselves. The parallel control group comprised sixty-two campers who never received sorbitol, while the remaining 143 campers made up the study group. No use was made of observations and measurements recorded during the initial three days of the camp or during the day of discharge, these days being considered as especially variable and required for adjustment to the routine of camp life. The remaining stay of each camper was divided into three periods: the *initial* period of between two and four days considered the necessary minimum for establishing a valid

baseline, the *intermediate* period ranging from eight to forty-eight days, which was the period of sorbitol administration in the case of the 143 campers of the study group; and the *final* period, usually of five days' duration during which, again, no sorbitol was given (i.e., the sequential control period within each group).

Whereas the daily insulin dose and the twenty-four-hour excretion of glucose were available in all campers, sixty-four campers were selected for the blood studies; twenty-two of these were assigned to the control group and forty-two to the group to receive sorbitol. Age and duration of diabetes were comparable in both the control group and the study group. The blood samples were obtained on the last day of each of the three camp periods, i.e., on the second or fourth day of the initial period; on the eighth, eighteenth, thirty-first or forty-eighth day of the intermediate period; and on the fifth day of the final period. Those campers who received sorbitol for longer than eight days had blood samples drawn more frequently, usually on four separate occasions.

*Mode of sorbitol administration.* The sorbitol employed was a colorless syrup of 70 per cent (weight for weight) sorbitol in water. Thus, since the specific gravity of the syrup was 1.3, each 10 ml. of sorbitol contained 9.1 gm. of sorbitol. Because of the side effects of sorbitol given orally in large amounts, i.e., abdominal cramps and diarrhea, the following rules were adhered to strictly. First, sorbitol was given at meal time only, mixed with food, such as in milk, on cereals, or on desserts. Second, the administration of sorbitol was initiated cautiously, only 5 ml. (one teaspoon) being given the first day, with subsequent gradual increase within five days to the maintenance dose of 45 ml. per day (one tablespoon with each meal). No adjustment was necessary for the age of the campers. The detailed initial program of administration is shown in table 3.

## RESULTS

*Insulin requirements and glycosuria in 143 campers given sorbitol, compared with sixty-two campers in the control group.* The over-all results are summarized in figure 4. For the purposes of this comparison, the mean values and standard errors for the daily insulin requirement and the daily glycosuria throughout the initial, intermediate, and final periods were calculated for each of the two groups. It is evident from the figure that a significant difference between the sorbitol group and the control group was not observed, and that in both instances the pattern was the same, i.e., increased insulin requirement and decreased glycosuria during the first half of the camp, with little further change thereafter. Whatever weight might be attached to the higher mean glycosuria in the sorbitol group during the intermediate period is offset by the lower mean insulin dosage during that time. Decreasing glycosuria and increasing insulin requirements during the early stages of the camp are to be expected and reflect the somewhat more aggressive therapy provided during the camp.

TABLE 3

Schedule of sorbitol administration

| Day of sorbitol administration | Amount of syrup (70 per cent weight for weight) | Grams per day |
|---|---|---|
| 1 | 5 ml. with breakfast | 4.5 |
| 2 | 5 ml. each with breakfast and supper | 9 |
| 3 | 5 ml. each with breakfast, lunch and supper | 13.5 |
| 4 | 10 ml. each with breakfast, lunch and supper | 27 |
| 5-48 | 15 ml. each with breakfast, lunch and supper | 41 |

INSULIN THERAPY AND GLYCOSURIA OF 143 CAMPERS WHO WERE GIVEN SORBITOL DURING THE INTERMEDIATE PERIOD, COMPARED WITH THE CONTROL GROUP (62 CAMPERS)*

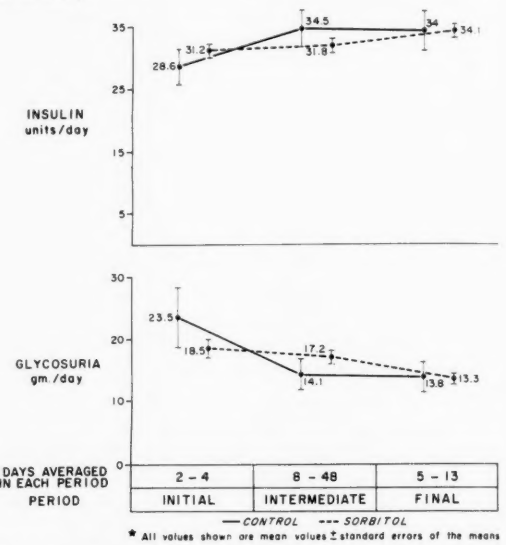


FIG. 4. Insulin therapy and glycosuria of 143 campers who were given sorbitol during the intermediate period, compared with sixty-two campers in the control group.

*Insulin requirement, glycosuria, and fasting blood measurements in forty-two campers given sorbitol, compared with twenty-two campers in the control group.* Results of the more detailed studies carried out in the smaller group of campers are shown in figure 5. As in the larger group, the values for insulin requirement and urinary glucose secretion overlapped for the initial, the intermediate, and the final periods. Furthermore, there was no significant difference between the mean values for the fasting blood glucose, the fasting blood ketone bodies, and the fasting plasma free fatty acids. In the case of the fasting blood ketone bodies and the fasting plasma free fatty acids, it cannot be firmly stated that the difference between the two groups might not have become significant with a larger number of observations.

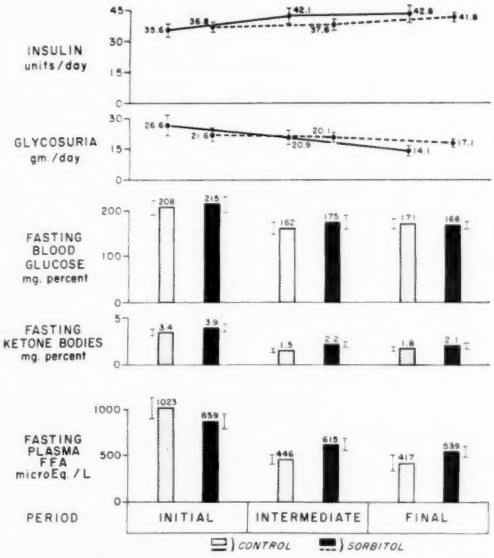


FIG. 5. Insulin therapy, glycosuria and fasting levels of blood glucose, blood ketone bodies, and plasma free fatty acids of forty-two campers given sorbitol during the intermediate period, compared with twenty-two campers in the control group.

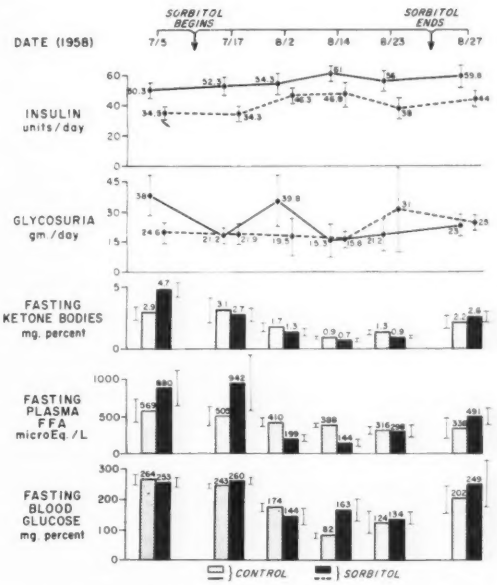


FIG. 6. Studies in nine campers who received sorbitol for forty-eight days, compared with six campers serving as controls.

However, it should be noted that the same trend persisted during the final period, i.e., after discontinuation of sorbitol administration. In the smaller group described below, members of which received sorbitol for a longer period, this difference was not seen.

*Observations in nine campers given sorbitol during forty-eighty days, compared with six campers in the control group.* Although the results shown in figure 6 concern a small group of campers, they are of special importance because they represent data from campers in whom a prolonged study was possible. The period of sorbitol administration was subdivided into four periods of 8, 16, 12 and 11 days, respectively, with blood samples obtained at the end of each of these periods. The mean insulin requirements of these two small groups significantly differed both on and off sorbitol, although the trend to gradually increased insulin dosage during the camp season was present in both. Glycosuria did not differ significantly for the two groups throughout the study, nor was there a significant difference in the fasting values for blood ketone bodies, plasma free fatty acids, and fasting blood glucose, except for the fasting blood glucose values obtained on August 14. It is of interest to note that, in this smaller group, the trend toward higher fasting blood ketone bodies and higher fasting plasma free fatty acids during sorbitol administration was not confirmed. In most instances the mean values for the sorbitol group were slightly less than those for the control group, with the exception of the plasma free fatty acids on July 17.

*Side effects.* Transient abdominal cramps were complained of by fifteen children (10 per cent) of the sorbitol group, as opposed to four children (6 per cent) of the control group. Diarrhea occurred in eighteen boys (12.5 per cent) of the sorbitol group, and in thirteen (21 per cent) of the control group. In no instances were the symptoms sufficient to warrant the discontinuation of sorbitol. Kaopectate or Donnagel were used as temporary treatment, but never for more than two days. No other undesirable side effects were observed.

The campers received sorbitol enthusiastically. When directly questioned 139 (97 per cent) of the sorbitol group expressed their great liking for its sweet taste, while two "didn't care either way," and two "hated it." None of these last four had suffered from its undesirable side effects. Boys from the control group frequently requested that they be given sorbitol also.

Sorbitol has the same caloric value as any other carbohydrate and the maintenance dose of 41 gm. thus amount to 164 extra calories. Since this was added to the prescribed diet it is likely that weight gain would follow, if observations could be made over a sufficient

period of time. The duration of the camp was too short for the occurrence of a significant difference in the final weights between the sorbitol receiving group and the control group.

The slow absorption of a small amount of sorbitol from the gastrointestinal tract might provide the liver with a steady, though small, amount of glucose precursors, and thus might decrease the incidence of insulin reactions, or at least reduce their severity. However, there was no change in the incidence of reactions and when questioned as to the severity of the reactions, 108 campers (76 per cent) indicated no change in the usual pattern, twenty-five campers (17 per cent) thought their reactions were either less severe or preceded by better warning symptoms, while ten boys (7 per cent) reported a paradoxical effect from sorbitol, with increasing severity and less readily perceived warning symptoms.

## DISCUSSION

It would appear from these studies that sorbitol may be administered in quantities as great as 41 gm. per day, *in three divided doses with meals,* to juvenile diabetics without significant incidence of undesirable side effects. This does not appear to be associated with an increase in insulin requirement as judged by the insulin dose thought to be necessary by the treating physician, the amount of glucose excreted in the urine, and fasting values for blood glucose, blood ketone bodies and plasma free fatty acids. This conclusion is in agreement with that made first by Thannhauser in 1929,[47] by others since, including most recently Mehnert and his collaborators in Munich.[39] None of these studies, however, has been carried out in a large number of patients with the *juvenile* form of diabetes. That very large quantities of sorbitol or fructose may be retained by the adult type of diabetic has been established by means of intravenous infusions over eight hours by Felber and coworkers,[31] and by means of prolonged intragastric instillations by Moorhouse and Kark.[30] The possibility that sorbitol might not be absorbed from the intestinal tract to any significant degree, and that its metabolism might primarily be a bacterial one, has become untenable since the studies of Adcock and Gray[35] who found that single oral doses as large as 35 gm. completely disappeared from the gastrointestinal tract, both with and without concurrent intestinal antibacterial therapy. Adcock and Gray further supported their conclusions with studies of the metabolism of orally administered sorbitol labeled with C-14. The rates of absorption for sorbitol measured in the human intestinal tract by Mehnert et al.[48] are quite sufficient to account for complete absorption of small doses such as have been administered in this study, unless diarrhea is present. The data shown in figure 7, indicating a parallel between amounts of sorbitol given orally and those excreted in the urine, further suggest the concept of intestinal absorption of sorbitol proportional to the administered dose.

TWENTY-FOUR-HOUR EXCRETION OF SORBITOL ON DIFFERENT SORBITOL DOSAGES

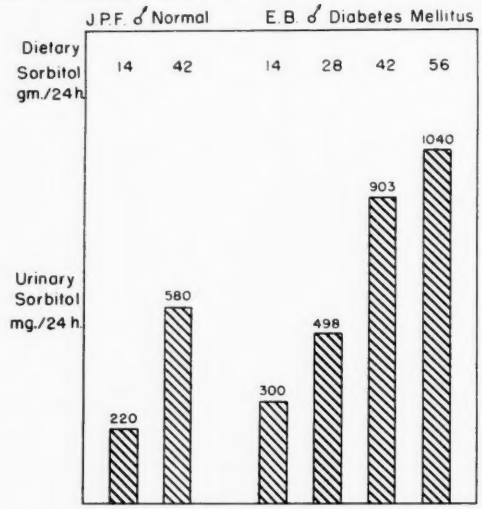


FIG. 7. Twenty-four-hour excretion of sorbitol on different dosages of sorbitol by mouth. Unpublished observations of Felber and Renold. Sorbitol was measured by a modification of the method of West and Rapoport.[55]

Whereas the observations reported here suggest that quantities of sorbitol as great as 41 gm. per day can be added to the carbohydrate intake of juvenile diabetics without significantly altering the insulin requirement, it is apparent from the results that the administration of sorbitol failed to be accompanied by detectable improvement in any of the measurements selected as indices of metabolic control. This would appear not to support the rationale outlined above for the possible usefulness of sorbitol in diabetics. However, it should be clearly understood that the lack of positive evidence in this instance cannot be considered as conclusive. Several possible reasons come to mind. First, it is evident that the indices of metabolic control selected may not have included the most appropriate ones since they primarily represented acute indices. In future studies it would be desirable to include also, particularly in the case of prolonged periods of administration, metabolic indices which are less labile, such as serum cholesterol, serum protein and particularly lipoprotein patterns, and possibly serum glycoprotein components. Secondly, the time

at which the more sensitive indices, such as plasma free fatty acids and blood ketone bodies were obtained may not have been optimal. For practical reasons, a late evening dose of sorbitol was not administered, resulting in a sorbitol-free interval from 5:30 p.m. (suppertime) until 7:00 a.m. when the blood samples were obtained. Even if the slow rate of intestinal absorption is taken into consideration, it is evident that significant absorption must have ceased many hours prior to obtaining the fasting blood. In subsequent studies, provision for a late evening dose should be made.

*In conclusion*, therefore, it would seem that sorbitol administration, with the precautions observed here, is a means of increasing dietary carbohydrate in juvenile diabetics by as much as 40 gm. daily without increasing the insulin dosage and without untoward side effects. In so doing the sweetness of sorbitol may be taken advantage of, a feature of special appeal to children. Although the caloric value of sorbitol has to be considered, this is not likely to represent a major problem in juvenile diabetes in whom the tendency to overweight is not great. If overweight is present, sorbitol may be substituted for dietary fat. While the resulting slow but persistent availability of carbohydrate not requiring insulin for its hepatic and renal utilization appears advantageous for theoretical reasons as outlined above, objective changes of indices of carbohydrate and lipid metabolism have not been detected. Continued studies of the possible usefulness of sorbitol and related substances, such as fructose, in larger series of patients, using prolonged periods of administration, and testing a variety of metabolic indices, are indicated. Although the cumbersome nature of such studies is fully appreciated, justification derives in part from the present paucity of hopeful leads for the further practical improvement of the still imperfect therapy of juvenile diabetes.

## SUMMARY

The metabolism of fructose and sorbitol has been reviewed and an attempt made to evaluate the possible usefulness of sorbitol as an adjunct to the therapy of diabetes mellitus in children and young adults. The rationale for this therapy is based on the possible metabolic benefit to be derived from (1) the availability to hepatic metabolism of a hexose (fructose) of which the initial phosphorylation is not insulin dependent, and (2) the availability to all tissues of metabolites of this hexose, such as lactate and pyruvate. Sorbitol may be preferable to fructose because of the apparent lack of sorbitol metabolism in the course of intestinal absorption. While the slow rate of intestinal absorption of sorbitol accounts for its laxative effect at dose levels in excess of 50 gm. per day, it also may be of special usefulness in continuously providing small amounts of carbohydrate to hepatic metabolism throughout a large portion of the twenty-four-hour period.

Sorbitol was added to the diet of 143 of 205 boys at a summer camp, for periods varying from eight to forty-eight days. The period of sorbitol administration was preceded and followed by a control period and, furthermore, sixty-two campers in parallel control groups never received sorbitol. Up to 40 gm. sorbitol daily, given in three daily doses during meals, was tolerated without gastrointestinal side effects and without evidence of increasing insulin requirement. Glycosuria and daily insulin dose, recorded in all campers, and fasting blood values for glucose, ketone bodies and free fatty acids, obtained at regular intervals in sixty-four boys, did not appear to vary significantly as a result of the sorbitol added to the diet.

## ACKNOWLEDGMENT

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## SUMMARIO IN INTERLINGUA

*Le Evalutation de Sorbitol in le Dieta de Juvenes Diabetic in Campings de Estate*

Es revistate le metabolismo de fructosa e sorbitol. Es facite un essayo de evalutar le possibile utilitate de sorbitol como adjuncto al therapia de diabete mellite in juvenes e juvene adultos. Le argumento in favor de iste therapia signala le possibilitate de un beneficio metabolic resultante de (1) le disponibilitate al metabolismo hepatic de un hexosa (fructosa) con un phosphorylation initial que non depende de insulina e (2) le disponibilitate a omne tissus de metabolitos de iste hexosa, incluse lactato e pyruvato. Il es possibile que sorbitol es preferibile a fructosa a causa del facto que (apparentemente) in le curso del absorption intestinal de sorbitol il non occurre un metabolismo de illo. Durante que le lente absorption intestinal de sorbitol explica su effecto laxative a nivellos de dosage in excesso de 50 g per die, il etiam pare possibile que iste proprietate del droga rende lo particularmente utile in tanto que illo provide micre quantitates de hydrato de carbon pro le metabolismo hepatic durante un considerabile portion del periodo de vinti-quatro horas.

Sorbitol esseva addite al dieta de 143 inter 205 pueros in camping estival durante periodos de inter octo e quaranta-octo dies. Le periodo del administration de

sorbitol esseva precedite e sequite per un periodo de controlo. In plus, sexanta-duo altere pueros participante in le camping nunquam recipeva sorbitol e esseva observate como gruppos de controlo. Usque a 40 g de sorbitol per die, administrate in tres doses diurne durante le repastos, esseva tolerate sin adverse effectos lateral in le systema gastrointestinal e sin evidentia de un augmento del requirimento pro insulina. Le glycosuria e le dosage diurne de insulina (que esseva notate pro omne le participantes del campings) e le valores sanguinee in stato jejun pro glucosa, corpores cetonic, e libere acidos grassiose (que esseva obtenite a intervallos regular in sexanta-quatro pueros) non pareva variar significativemente in consequentia del addition de sorbitol al dieta.

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## *Diabetes Mellitus and Pyelonephritis*

It has become abundantly clear, in the past few years, that asymptomatic infections of the urinary tract can be recognized by the proper use of quantitative bacteriologic methods, and that asymptomatic bacteriuria not only may accompany pyelonephritis but also may lead to the development of symptomatic pyelonephritis. Studies of the incidence and significance of bacteriuria have been greatly aided by the use of voided urine for bacteriologic study, and the data so obtained have suggested that asymptomatic bacteriuria is a common infection, with potential consequences that require careful study.

Many attempts to define the incidence of bacteriuria in various clinical states, such as diabetes mellitus, hypertension and pregnancy, are in progress. As is often the case, the application of a different approach to old problems has also provided a stimulus to examine critically some traditional concepts. Huvos and Rocha, in work reported elsewhere in this issue (of *The New England Journal of Medicine*), have studied the incidence of bacteriuria in hospitalized patients with diabetes mellitus. Their findings that there was little difference between diabetic and carefully matched nondiabetic patients raises important questions.

Pyelonephritis has long been recognized to be a common and often severe complication of diabetes mellitus. It is generally believed that pyelonephritis and other infections occur more frequently among diabetic than among nondiabetic patients. This belief has been taken as evidence to support the hypothesis that metabolic disturbances in diabetes mellitus influence directly mechanisms of resistance to infection.

The question is now posed, how sound is the evidence that infections occur especially often among diabetic patients and that this reflects a defect in immunity? The usual clinical experiences must be regarded as inadequate to answer this question directly. There can be little doubt that circumscribed and "minor" infections may aggravate pre-existing diabetes, and thus call attention to the presence of infection. It is likely that this added threat to survival has led diabetic patients more frequently to physicians, and has led physicians, in turn, to a more assiduous search for infections in diabetic patients.

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# Progressive Ocular Myopathy with Ovarian Insufficiency and Diabetes Mellitus

## Report of a Case

*Mervyn Lakin, M.D., and Simeon Locke, M.D., Boston*

"Progressive nuclear ophthalmoplegia" or "abiotrophic ophthalmoplegia externa" has been established recently as an ocular myopathy on the basis of biopsy of extraocular muscles.[1-4] Earlier, the clinical picture and the gross and microscopic appearance of the levator palpebrae superioris, led Fuchs, cited by Kiloh and Nevin,[1] Collins,[5] and Martin,[9] to suggest the dystrophic nature of this condition. It has been recorded in familial[7-12] and sporadic forms,[13-15] in association with dystrophia myotonica[1] and in limb girdle dystrophy.[14-16] The opportunity for extensive study of a patient with proved ocular myopathy and an associated endocrine deficiency provided an occasion to re-evaluate the process and report this unique association.

### CASE REPORT

A sixty-year-old, unmarried, white woman was admitted to the New England Deaconess Hospital with bilateral ptosis, external ophthalmoplegia, and weakness of the legs. Ptosis had first been noted at age twenty-five. Because of insidious progression, bilateral corrective surgery was performed in 1933 and again in 1950. Increasing difficulty in walking had been noted during the year prior to admission.

The patient was the oldest of six children. Three brothers and one sister are in good health. One brother died at age fifty-seven years of cancer of the stomach. Her parents died at ages seventy-eight and fifty-six of cardiovascular disease and cancer. There was no family history of ocular or muscle disease. Two siblings were examined personally and were free of extraocular palsy. Photographs of the remaining siblings, the parents, and the patient at age nine revealed no ptosis.

In 1952 the patient was discovered to have diabetes mellitus. At that time extensive endocrinological evaluation revealed that there had never been menstrual bleeding and that breast development had not occurred. There was no history of mumps. Urinary FSH was positive at 104 mouse units per twenty-four hours. A diagnosis of ovarian insufficiency of undetermined etiology was made. There was striking osteoporosis and fractures of both hips, both wrists and the left humerus had occurred between 1946 and 1952. Height had decreased from 61 inches to 58½ inches. On neurological examination, there was slight weakness of the thighs and weakness of dorsiflexion of the feet, more marked on the right. Upper limb strength was normal. Vibration and pain perception was decreased below the knees bilaterally. In 1952 she was started on diet and insulin for her diabetes, and on stilbestrol for osteoporosis. Withdrawal of stilbestrol produced uterine bleeding. Between 1952 and the current hospitalization several episodes of severe depression occurred and a course of electroconvulsive therapy was administered in 1953.

On direct questioning during the current admission, the patient admitted to blurring of vision without frank diplopia, intermittent huskiness of the voice, occasional mild dysphagia, and cold intolerance.

Examination revealed her to be obese and obviously depressed. Pulse was 108, and blood pressure was 205/120. There were early senile lenticular opacities. Grade II arteriosclerotic changes, occasional microaneurysms, and striate hemorrhages were noted on fundoscopic examination. The thyroid was not palpable. There was marked kyphoscoliosis and a grade II blowing systolic murmur was heard along the left sternal border. Breasts were underdeveloped but hair distribution was normal. No abdominal organs were palpable. There was striking bilateral ptosis (figure 1) and extraocular movements were restricted to five degrees in all directions. Pupils were 3 mm. each and reacted to light and on accommodation. There was wasting of the temporalis muscles with hollowing of the temporal fossae. Bilateral facial weakness involved the upper and lower face. The sternocleidomastoid muscles were conspicuously weak and wasted bilaterally, the left more markedly than the right. Gait was unstable and shuffling. Distal wasting and weakness of the arms and hands and less striking weakness of the distal muscles of the legs were noted. The muscles of the shoulder girdle were normal. There was weakness of hip flexion bilaterally. The biceps reflexes were markedly depressed and tendon reflexes were absent elsewhere. Plantar response was bilaterally flexor. Perception of pain and cold was decreased on the feet and lower legs. Percussion myotonia was not present even following immersion of the hand in cold water for thirty minutes. Tensilon test was negative.

Hemogram and urinalysis were normal. Blood chemistries revealed: BUN 17.5 mg. per cent; cephalin-cholesterol flocculation 1+ at forty-eight hours; Hinton negative; cholesterol 197 mg. per cent; blood sugar 200 mg. per 100 ml. Electrocardiogram showed complete right bundle branch block and a remote posterior myocardial infarction. Endocrinological evaluation revealed: B.M.R. + 24 per cent, PBI 6.0 μg. per cent; total iodide 6.5 μg. per cent; twenty-four-hour RAI uptake 28 per cent; twenty-four-hour 17-hydroxysteroids 4.5 mg., and 10.3 mg./24 hr. (normal 0.8-8.5 mg./24 hr.); 17-ketosteroids 8.9 and 7.5

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FIG. 1. Facial appearance of patient. Note hollowing of temporal fossae, facial weakness and atrophy of sternocleidomastoids.

mg./24 hr. (normal value 6.5-17.4 mg./24 hr.), FSH positive to 20 and negative to 50 mouse units/24 hr. on one specimen and positive to 50 and negative to 100 mouse units/24 hr. on a second specimen (normal postmenopausal female positive to 50 and negative to 100 mouse units/24 hr.). A buccal smear disclosed a female chromatin pattern. Skull films showed a normal pituitary fossa.

Serum calcium varied between 9.5 and 10.1 mg. per cent, and serum phosphorus between 4.2 and 5.0 mg. per cent. After a 200 mg. calcium diet for three days, the twenty-four-hour calcium excretions ranged from 92 to 154 mg. and the twenty-four-hour phosphorus excretions ranged from 456 to 558 mg. The total phosphorus reabsorption was 92 per cent. The alkaline phosphatase was 3.8 B.U. X rays of the spine and feet revealed marked osteoporosis with several biconcave deformities of the vertebrae.

Laboratory evaluation of the muscular disorder revealed: Serum potassium 4.4 mEq./L.; serum magnesium 2.1 mEq./L.; transaminase 47 units; serum creatinine 0.6 mg. per cent; twenty-four-hour creatinine clearance 95 cc./min.; twenty-four-hour urine potassium 65 mEq., and twenty-four-hour urine creatine 95 mg. Lumbar puncture produced clear, colorless, acellular fluid under normal pressure with a protein of 23 mg. per cent, Cl of 129 mEq./L., negative serology, and colloid gold of $0^{10}$.

Electromyography revealed a typical myopathic pattern in the right medial and right lateral rectus muscles. Myotonia was not present in eye or peripheral muscles. Peripheral nerve conduction velocities were: right ulnar nerve 64 m./second; right median nerve 53 m./second, and right peroneal nerve 46 m./second. Biopsy of the right lateral rectus muscle was performed (figure 2). Pathological report states: "This is a fragmented bit of muscle which was embedded in paraffin and stained by hematoxylin and eosin, phosphotungstic acid hematoxylin, Mallory's aniline blue, Verhoeff's elastic tissue stain and periodic acid Schiff. There is a striking increase in endomysial connective tissue and a definite variation in fiber size within a single fasciculus. The nerve endings that are seen appear normal. There are several free polymorphonuclear cells about a blood vessel and occasionally free in the tissue. This may be related to the electromyographic trauma prior to the biopsy. The changes are consistent with a myopathy." Permission for biopsy of a peripheral muscle was refused.

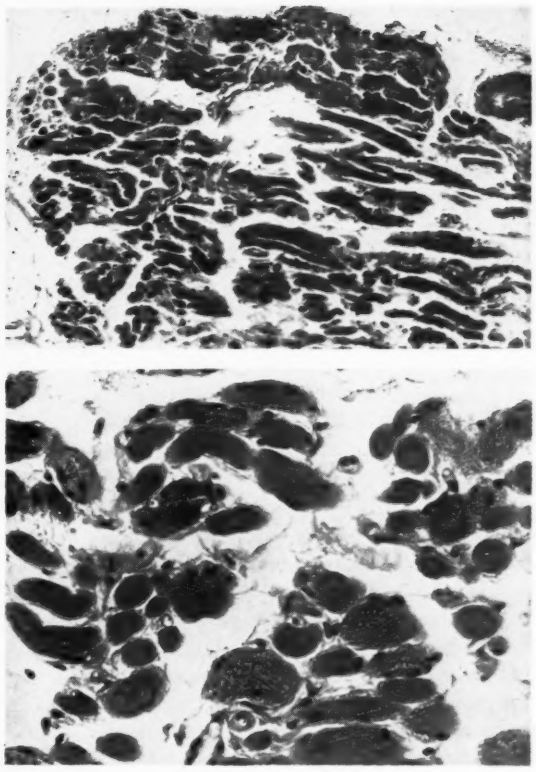

FIG. 2. Histological appearance of right lateral rectus muscle. Top 103X, bottom 412X. Note increase of endomysial tissue and variation of fiber size.

Because of increasing intensity of depression, the patient was started on phenylethylhydrazine (Nardil $R_X$) 15 mg. t.i.d. Shortly after institution of therapy a fluctuating rigidity of the extremities was noted. On termination of therapy marked clearing of rigidity appeared. Increased depth of depression with some associated paranoid ideas made management in a general hospital difficult and the patient was transferred to another institution.

## DISCUSSION

Myopathy of the extraocular muscles is well documented in disorders which also affect the facial muscles, sternocleidomastoids, and muscles of the girdles.[14-16] It has been reported in the absence of myotonia,[17] in distal myopathy,[2] and in dystrophia myotonica.[3,4] Langdon and Cadwalader[18] and Delord[8] suggested a neural basis for chronic progressive external ophthalmoplegia but no histological examination of extraocular muscles was per-

formed. In retrospect, many of the cases in which a nuclear origin was initially predicated probably resulted from primary disease of muscle. This is suggested by case II of McMullen and Hines,[19] in which subsequent examination by Gordon Holmes revealed "weakness of various muscles of limbs and face."

Endocrine dysfunction in association with ocular myopathy is rare. Bell[20] commented on the occasional association of muscular dystrophy and "dyspituitarism," and a recent report[21] described gynecomastia in a twenty-three-year-old male with scapulohumeral dystrophy and ophthalmoplegia. Sterling[22] reported an eleven-year-old boy with initial weakness and atrophy of the arms followed by involvement of the trunk and legs. Ptosis had been present since childhood. An elder sister had Charcot-Marie-Tooth disease. The patient had an aplastic left testicle, Freidreich's deformity of the feet, impairment of upward gaze, and absent reflexes without fibrillation or reaction of degeneration. Sterling felt he was dealing with an atypical form of Landouzy-Dejerine dystrophy because of the ophthalmoplegia and gonadal aplasia.

In the present case, the association of ocular myopathy with ptosis, facial weakness, wasting of the temporalis and sternocleidomastoid muscles, distal limb weakness and wasting, and affection of the endocrine system is reminiscent of dystrophia myotonica, despite the absence of myotonia. Martin[17] also noted this similarity in a twenty-seven-year-old male with an external ophthalmoplegia and myopathy. Extraocular muscle palsies are more frequently reported in myotonia congenita than in the dystrophic form of the disorder,[23] although the two diseases have been considered as varying manifestations of a single process.[24] In the absence of family history of the disorder, frontal baldness or posterior polar cataracts, the clinical picture in the patient reported is not entirely typical of dystrophia myotonica. More important, however, is the lack of clinical or electromyographic evidence of myotonia or paramyotonia. Although the electrocardiographic findings are similar to those found in dystrophic heart disease they may be explained on the basis of arteriosclerotic heart disease and be completely unrelated to the myopathy.

In the present case, ovarian insufficiency and diabetes mellitus could be construed as manifestations of a "pluriglandular affection" associated with a disorder of muscle. In that sense the findings in this patient may be of importance in providing a better understanding of the association of a genetically induced myopathy with concomitant disease of other organ systems, and may represent an atypical "forme fruste" of dystrophia myotonica.

## CONCLUSION

A case of a sixty-year-old woman with ocular myopathy proved by electromyography and biopsy is reported. In addition, diabetes mellitus and ovarian insufficiency were present. The literature on ocular myopathy in relation to dystrophic disorders of muscle is reviewed briefly.

## SUMMARIO IN INTERLINGUA

*Progressive Myopathia Ocular, con Insufficientia Ovarian e Diabete Mellite: Reporto de un Caso*

Es reportate un caso de myopathia ocular provate per electromyographia e biopsia in un femina de sexanta annos de etate. In plus, diabete mellite e insufficientia ovarian esseva presente. Es presentate un breve revista del litteratura concernite con myopathia ocular in relation a disordines dystrophic de musculo.

## ACKNOWLEDGMENT

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## *Diabetes Mellitus and Pyelonephritis*

When diabetes mellitus has been produced experimentally with alloxan or by pancreatectomy, no significant alteration in resistance to administered pathogens has been observed. Hamsters with spontaneous diabetes mellitus give little morphologic evidence of the presence of infection. Only during the acute toxic stage that occurs shortly after the administration of alloxan to rabbits has there been a demonstrable decrease in resistance to infection, and the toxic, rather than the diabetogenic, effect of alloxan may well have been responsible.

At autopsy, according to Robbins and Tucker, the incidence of pneumonia and of other infections was about the same in diabetic as in nondiabetic patients, but pyelonephritis occurred more frequently among the former. More recently, Kass reported that bacteriuria was about three times as common among outpatient women in the diabetic clinic as in the medical clinic, but the control aspects of this study, as in that of Robbins and Tucker, were not so carefully delineated as those of Huvos and Rocha. It is apparent, then, that infections are a greater hazard to diabetic than to nondiabetic patients, but not yet clear whether there is an increased incidence of infection among the former. It is clear that hospitalized populations, whether or not diabetic, have an astonishingly high incidence of bacteriuria, and an equally noteworthy incidence of previous manipulation of the urinary tract.

To detect whether diabetic patients have more infection of the urinary tract than nondiabetic subjects, it may be necessary to study unselected, nonhospitalized population groups and to obtain control data from comparable population groups in which even nondiabetic persons with family histories of diabetes have been segregated. If a higher incidence of infections of the urinary tract is found in diabetic patients, the reasons may not relate to impaired immune responses in the host. The role of catheterization and similar manipulation of the urinary tract, of diabetic neuropathy affecting the bladder and of vascular renal disease with scarring, as well as of other related factors, requires scrutiny.

The relatively circumscribed problem of the incidence of bacteriuria in diabetes mellitus, therefore, has many dimensions. Its solution may lie, in common with many other important clinical problems, in the study of defined, unselected population groups. Conversely, investigation of the mechanisms by which diseases influence the host will be hampered unless clear and objective statements of the natural history of the diseases under scrutiny are available. As the study of disease moves from hospitals and clinics into relatively asymptomatic populations, it may be expected that better knowledge of the natural history of many diseases will emerge, and therapeutic, prophylactic and mechanistic explorations will become more fruitful.

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# EDITORIAL

## HUMORAL NATURE OF THE HYPOGLYCEMIC FACTOR OF MUSCULAR WORK

The striated muscle mass of the body, by virtue of its weight alone, constitutes the major quantitative factor in the bodily conversion of foodstuffs to $CO_2$, water and energy. The flow of gluconeogenic precursors to the liver, the hepatic manufacture of sugar and the release of glucose in the satisfaction of the ever-changing needs of total muscular activity of the body represent a complex of regulation which is taken for granted but is understood inadequately in detail. Outside of an occasional epinephrine-induced hyperglycemia, the stability of the blood sugar gives no indication of the changing ebb and flow of foodstuff which attends the wide range of activity from the sedentary, basal muscle tone of deep sleep to the mild, moderate or severe exertions which characterize the normal course of events. The minute by minute alterations in oxygen consumption and the difficulties experienced in making a truly basal metabolic rate measurement are common clinical experiences. This variability in oxygen consumption must be reflected in equivalent variation in the rate of substrate metabolism.

In the control and regulation of a brittle, juvenile diabetic, a profound influence of muscular activity upon carbohydrate regulation is often dramatically evident. The lability of the diabetes of young patients yields to the strict dietary and insulin control of a hospital regimen only to be complicated by recurrent episodes of hypoglycemia with return to situations of exercise and play at home. The necessity for reducing insulin therapy with restoration of full range of muscular activity in the labile diabetic has long been recognized. Indeed, the capacity of muscular exercise to effect glucose metabolism in the complete absence of insulin has been systemically studied and demonstrated in animal experimentation.[1]

The insulin-independent capacity of muscular work to provoke increased glucose utilization and, indeed, to produce hypoglycemia in the diabetic organism has been attributed directly to the increased rates of metabolic processes associated with exercise. It has been logically assumed that with augmented rates of enzymatic degradation of glucose to $CO_2$, water, heat and contractile energy, a "vacuum" is created into which glucose "flows." In this fashion, a greater movement of glucose from the blood stream into the metabolic processes is envisioned. With the development of the "Glucose Transport Theory" of insulin action, this view concerning the hypoglycemic effect of muscular work becomes untenable.[2,3]

Initially with nonutilizable congeners of glucose and subsequently with more direct measurements of glucose, it could be demonstrated that insulin exerted a primary effect on promoting entry of glucose across a limiting cell surface into the cell interior. One could view the enzyme apparatus within the cell as ready and capable of handling large amounts of glucose in metabolic transformation but with cellular entry itself as the limiting step in metabolism of the sugar and the point of regulation by insulin. A distinctive apparatus for transporting glucose across this entry barrier was postulated, a transport apparatus which was insulin-dependent.

Almost from the beginning of our efforts along such lines, we recognized that such a functional and structural view of the cell in relation to the regulation of glucose metabolism was incompatible with the current notion of the effect of muscle work.[4] If entry of glucose is viewed as the limiting step at rest it must also be viewed as even more limiting during the conditions of augmented metabolic transformation of glucose in exercise. This theoretical discrepancy was subjected to study by parallel experiments with those used in devising the glucose transport theory of insulin action. It was found that induced muscular exercise promoted increased cellular entry of glucose and nonutilizable congeners of glucose.

In some fashion muscular exercise stimulated the transport of sugars across the cellular entry barrier and the interior of the cell now became available for occupancy by additional sugar. For glucose, the increased cellular entry of the sugar is induced by this effect of work at precisely the time when the enzyme apparatus is dissimulating the substrate at greater rates in meeting the energy requirements of exercise. Increased glucose metabolism of exercise becomes a secondary consequence of increased rates of entry, in a manner quite analogous to insulin, rather than a direct consequence of the augmented rate of glucose dissimilation by contracting muscle cells. The glucose transport theory is extended by

this view to be subject to at least two stimulatory factors, namely insulin and the effects of muscle work.

It was noted during these early experiments with induced muscular work that the nonutilizable congeners of glucose were distributed in a calculated space far greater than could be accounted for by the mass of muscles set into exercise. Indeed, with time, distribution of these nonutilizable sugars reached a calculated space of total body water and further work or insulin was incapable of extending this distribution any further. Since only one or both hind limbs were stimulated in such experiments, a maximal space of some 30 per cent of the carcass was being affected directly by the exercise. The possibility of a general, humoral effect of exercise became apparent. Muscle groups, as well as whole hind-limb areas were isolated neurologically and, when stimulated strenuously enough, would effect a generalized distribution in the resting tissues as well as in the working tissues.[4]

Most recently, we have confirmed the humoral nature of the hypoglycemic effect under these conditions.[5] Both by cross-transfusion of blood and by the drainage of thoracic lymphatic fluid, the hypoglycemic properties of the working animal can be transferred to a resting preparation. In a variety of combinations, chronically depancreatized dogs as diabetic donors and recipients and intact dogs as normal donors and recipients have been cross-connected. There appears to be little question that with strenuous induced exercise one can enrich the body fluids of an animal with hypoglycemic properties which can then be transferred to a resting preparation without other concomitants of exercise such as *p*H shifts, hyperpnea, circulatory changes, etc.

A number of workers have confirmed this insulin-like effect of muscular work but as a local phenomenon involving the working musculature alone. We are prone to regard these differences as arising from a failure to employ the same intensity of induced muscle work. Both in time and in degree, the contracting muscle itself would be expected to exhibit the greatest glucose entry effect of exercise. It remains to be seen whether this generalized effect, which exhibits itself in a humoral character, is an artifact of experimental degrees of muscle work or a reflection of normal physiologic processes in severe exercise.

At this point, the ability to generalize the hypoglycemic effect of exercise into a humoral process has the practical value of an approach to isolating the factor on the one hand, and the speculative value of at least showing the capacity for developing such a humoral character. At least under certain stringent experimental conditions, the insulin-free, chronically diabetic organism has the internal capacity to promote increased glucose utilization and reduction of hyperglycemia to normal or subnormal levels.

Attempts to isolate the circulating hypoglycemic factor induced by severe exercise have been singularly unsuccessful to date, and one can say little of the possible nature of this material. In these frustrated attempts at identifying the humoral factor, we can at least be assured that it is not residual insulin released from some inactive binding in the tissues. Directly transferred blood or lymph from working to resting preparations exhibit active and long-lasting hypoglycemic properties. Attempts to store these biological fluids or to manipulate them in the conventional approaches to isolation of a biological agent have revealed the great lability of such a hypoglycemic factor. Within minutes in vitro the blood and lymph lose their hypoglycemic properties. These same fluids exhibit no striking capacity to destroy or inactivate added commercial insulin. It would appear that this muscle factor is a highly labile, probably short-lived material in transit which must either reach and fix to an active glucose transport site or within minutes lose its identity as a hypoglycemic agent.

There is intrinsic interest and possible practical value in pursuing the study of self-regulation of glucose uptake by muscles during exercise and the isolation and identification of this muscle product. Of more far-reaching consequence may be a re-evaluation of our precise knowledge of the regulation of flow of foodstuff between liver and the widely fluctuating needs of muscles in their minute by minute change in activity. We are dealing here with one point in this regulatory complex. The relevant questions which arise are as manifold as the willingness to re-examine the seemingly logical explanations now available. One can readily contrast two young individuals of equal nutritional status, growth and general health, with the exception that one is a normal individual and the other an insulin-treated juvenile diabetic. In the recently fasting state, both will exhibit normal blood sugar levels. Insulin, derived spontaneously in the normal and by administration in the diabetic, is serving the function of regulating and establishing normoglycemia. Exercise in the normal is attended by appropriate shift in gluconeogenesis and glucose release with maintenance of blood level. Exercise in the insulin treated diabetic results in hypoglycemia. The explanation of the hypoglycemia in the insulin treated diabetic is far from obvious. Did the normal individual reduce his insulin secretion below fasting basal levels with exercise or was the maintenance dose of insulin in the dia-

betic subcutaneous depot actually excessive to begin with? Does the juvenile diabetic exhibit a second functional lesion in the manner in which muscular exercise elicits and perhaps generalizes a hypoglycemic factor of work? Is the hepatic glucose release in exercise inadequate in the treated diabetic? The ability to regulate a condition per se with an agent like insulin does not compel the conclusion that the underlying defect must be sought exclusively in the inadequacy of insulin secretion.

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# *BOOK REVIEWS*

INTERNATIONAL BIGUANIDE-SYMPOSIUM (INTERNATIONALES BIGUANID-SYMPOSIUM) PROCEEDINGS OF CONFERENCE HELD MAY 12-13, 1960, AT AACHEN, GERMANY. *Edited by F. Bertram and G. Michael. $4.00, pp. 167, Georg Thieme Verlag, Stuttgart, Germany, 1960. (In U.S.A. and Canada, Intercontinental Book Corporation, New York 16, N.Y.)*

Though described as international, almost all of the participants of this "Biguanid-Symposium" were from German-speaking countries (Germany, Austria and Switzerland); only two of twenty-one papers were presented by American authors. This alone demonstrates the great interest and the wide acceptance which the hyperglycemic biguanides have found in Europe, particularly in Germany, which, thirty years ago, rejected as too toxic the first orally effective hypoglycemic agent, a guanidine derivative, introduced by the German clinician, Frank. Indeed, Professor Bertram of Hamburg who once had opposed the use of Synthalin because of disappointing clinical experiences, presided over the present symposium and stated in his concluding remarks: "that I would not like to overlook again biguanide in the therapy of diabetes mellitus." His opinion was based on the universally favorable reports from German and Austrian hospitals and clinics on the use of the biguanides as sole agents in the treatment of maturity onset diabetes and in combination with insulin in the treatment of growth onset and brittle diabetes. Besides DBI (phenformin) the German investigators employed a butyl-biguanide (W37). Reference was also made to a dimethyl biguanide (La 6032) apparently widely used in France. While these three agents seem to have about the same mode of action and the same side effects (mainly, gastrointestinal incompatability), they differ in their effective dosage. DBI appears to be twice as potent as butyl-biguanide and twenty times as much as the dimethyl derivative. None of these agents seems to have shown any organic toxicity when used in clinically recommended doses, but reference was made to a few instances of ketoacidosis, apparently precipitated by the compounds. Considerably larger doses caused marked pathological changes, particularly in the liver and kidneys of experimental animals. Therefore, Creutzfeld warned against premature optimism. Caution seems indeed to be warranted since the mechanism of action of the hypoglycemic biguanides is still poorly understood. The theoretical papers and discussions added much speculation but few new facts. The main paper in this section gave a summary of the research data of the various laboratories in America.

This well prepared volume contains transcripts of most of the discussions, in addition to the formal papers. It should be valuable to everyone interested in the modern management of diabetes.

PRACTICAL CLINICAL MANAGEMENT OF ELECTROLYTE DISORDERS. *By William J. Grace, M.D. $4.95, pp. 132, Appleton-Century-Crofts, Inc., New York.*

This is a small book, written in simple terms. In the preface, the author states that many monographs on fluid balance are capably prepared but contain too much detailed physiology for most physicians. In this book the author presents, by topic, clinical case histories first, then discusses briefly the management, along with physiology. This type of outline is in contrast to the usual book published on fluid balance.

There are ten chapters. The author starts with a discussion of alkalosis due to gastric fluid loss. Case reports are given with a discussion of physiology following. A liberal use of ammonium chloride in the treatment of alkalosis is recommended. However, no mention is made of the possible toxicity of ammonia. It might be said that the liberal use of ammonium chloride in the treatment of alkalosis as yet has not come to be accepted by all. Following, there is a discussion of heart failure with hyponatremia. The material is well presented. Next, uremia, renal acidosis and acute renal failure are discussed. There is a chapter on diabetic acidosis. Some authorities in the field of diabetes might disagree with the method of treatment presented. Dr. Grace gives only 50 units of insulin per hour because "the body cannot utilize more than 50 units of insullin per hour." This statement might be challenged. Also, most authorities would consider 6 to 8 liters of saline as an excessive amount of sodium input unless there was evidence of severe extracellular dehydration. Furthermore, 60 mEq. of potassium as an average replacement dose is insufficient.

The author then follows with a section on electrocardiographic changes in electrolyte abnormalities. Chapter Seven deals with the replacement of electrolyte deficits. The author appears to favor the use of formulas and calculations to decide amounts of replacement therapy. It is this reviewer's belief that formulas can never substitute for clinical experiments in deciding upon replacement therapy. However, the author does point out the limitations of each formula. A chapter dealing with the Henderson-Hasselbalch Equation is presented. It is clear and well outlined. Finally, the author concludes with sections on magnesium and potassium deficiency.

This book is very easy to understand. It should present no burden even to the medical student. It will serve as a guide to those who wish to obtain practical advice in the management of a patient, without getting lost in physiology. The small amount of physiology present in the book is put clearly. The points made by the author in the introduction are fulfilled.

DER DIABETES MELLITUS ALS VOLKSKRANKHEIT UND SEINE BEZIEHUNG ZUR ERNAHRUNG. *By Ernst-Günther Schenck and C. H. Mellinghoff. $13.50. pp. 310, Dr. Dietrich Steinkopff Verlag, Darmstadt, Germany, 1960.*

This book presents a statistical study of morbidity and mortality of diabetes, in and outside of Germany, and a review of nutritional factors which may contribute to the development of diabetes. The senior author leans heavily on the American studies of statistics of diabetes, especially those of Joslin and Wilkerson. For this reason, of more interest to us is what he has to say about diabetes statistics in Germany. Unfortunately, his data on incidence of diabetes in that country were obtained from statistics on death resulting from diabetes or from requests for special diets during the war. Such data do not compare favorably with those made available in the United States through our diabetes detection drives. Though of necessity not very reliable, the compilation of German statistical reports seems to indicate that diabetes morbidity and mortality vary greatly from one section of the country to another.

The question of diabetic diets in relation to degenerative complications of diabetes is discussed at length, but no conclusive evidence for or against the importance of nutritional factors in this area is presented. These uncertainties may possibly find their explanation in the anonymous replies to a questionnaire sent out by Parzran which reveal that scarcely 30 per cent of diabetic patients adhere to their prescribed diets. The decreased mortality from diabetes in Germany during the war is ascribed by the author to the use of diets which were low in fat and high in carbohydrate. It is of interest in this connection that fatalities among German diabetics during the war were due to infections in 38 per cent and to vascular complications in 35.6 per cent, while after the war, with a higher fat intake, fatalities from cardiac, vascular and renal complications of diabetes rose to 60 per cent of all diabetes fatalities. For these reasons the author advocates diets which supply three to four times more calories derived from carbohydrates than those derived from fat.

With increases in carbohydrate intake during the war the need for insulin also increased. Thus, doubling the carbohydrate intake from 125 to 250 gm. daily resulted in a 50 to 100 per cent increase in insulin requirement. To some extent this increased need for insulin could be alleviated by a reduction in fat intake.

The effects of starvation on the course of diabetes were observed during the war in countries under German occupation, such as Poland, France, Belgium, etc., in which starvation was deliberately imposed on the native populations. They consisted of spontaneous hypoglycemic convulsions and high sensitivity to even small doses of insulin. The reports on starvation in those countries mention fatal hypoglycemia unresponsive to glucose and severe posthypoglycemic brain damage often terminating in death. From the reviewed book, it appears that German writers became acquainted with such manifestations of starvation in diabetes some two years after the War (1947).

During the war Germany was cut off from meat supplies from South America, and of necessity had to switch to porcine pancreases for its production of insulin. It is of interest that a substantially better recovery of the hormone was obtained from pork than from beef or calf pancreases.

From the presented considerations the author concludes that there is a need for a socio-hygienic, prophylactic as well as a therapeutic, approach to diabetes. To satisfy this need, there exist in Germany counseling organizations which offer their cooperation to diabetic patients in hospitals and clinics.

The second part of the book is devoted to a lengthy discussion of diets used in diabetes, but there are no data to indicate the importance of nutritional factors in relation to diabetes morbidity and mortality or to the degenerative complications of diabetes. The dissertation abounds, however, in statements to which American workers in the field may take exception. For instance, the junior author states that diabetics should avoid white bread and eat chiefly dark bread because of its slow absorption from the gut (*p.* 228). Cakes are of course prohibited entirely, since they are made with white flour and sugar; oranges should be taken only as an exception because of their high sugar content, while beer should never be drunk because it contains 4 to 5 per cent of readily absorbable carbohydrates.

One also finds in this book such assertions as: that smoking may precipitate the onset of diabetes, that juvenile diabetics do not become overweight even on high calorie diets, that liver damage contributes to the development of vascular complications, and that thiamine deficiency may be the cause of insulin resistance. One seldom finds such an array of unwarranted statements in a scientific dissertation.

INSULIN, British Medical Bulletin. *$3.25, Volume 16, Number 3, published by the Medical Department, The British Council, 65 Davies Street, London W.1, September 1960.*

The September 1960 number of the British Medical Bulletin is devoted to a collection of succinct and authoritative essays on the chemistry of insulin and the physiology and biochemistry of insulin action. The problems of insulin and noninsulin hypoglycemic agents in practice are also discussed. The authors, who include the most distinguished British workers in the field, have produced models of precise and easy-reading scientific writing. Unlike many similar ventures, this collection seeems to have gone from manuscript to publication without the excessive delay that might have robbed it of its value as an account of the current status of the field. It is without exaggeration an invaluable addition to our literature on insulin.

# ABSTRACTS

*Ammerman, C. B.; Iacono, J. M.; Wendel, Lucille P.; and Isaac, G. J.* (U.S. Army Med. Res. & Nutrition Lab., Fitzsimmons Genl. Hosp., Denver, Colo.): EFFECTS OF CALORIC INTAKE ON CLEARANCE OF INJECTED LIPIDS IN RABBITS AND RATS. Am. J. Physiol. *200*:75-79, January 1961.

Rabbits fasted three and ten days displayed marked increase in serum total fatty acids and total cholesterol, limited increases in lipid phosphorus and a distinct inability to clear intravenously injected triglycerides from the serum. Limited feeding in which rabbits were losing weight over a ten-day period resulted in essentially no change, either in serum lipid components or lipid removal rates. The adverse effects of fasting on serum lipid clearance appeared to be partially overcome by injecting additional glucose at the time of lipid administration, and completely overcome by giving heparin intravenously fifteen minutes prior to lipid injection. Rats fasted four days showed decreased levels of serum total fatty acids, lipid phosphorus and total cholesterol, but maintained normal rates of serum lipid clearance. Limited-fed rats showed less pronounced decreases in serum total fatty acids, lipid phosphorus and total cholesterol, and also maintained normal serum lipid clearance. L.S.S.

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*Annegers, John H.* (Dept. of Physiol., Northwestern Univ. Medical School, Chicago, Ill.): NET ABSORPTION OF WATER, CHLORIDE AND HEXOSE FROM THE INTESTINE OF DOGS. Am. J. Physiol. *200*:107-10, January 1961.

Hexose and/or NaCl solutions were perfused at about 100 cm.$H_2O$ pressure through small intestinal loops in unanesthetized dogs. The rate of glucose absorption increased in curvilinear fashion as the glucose concentration of the infusate ranged from 1 per cent to 30 per cent and did not differ between jejunal and ileal loops. Net chloride secretion rates were independent of perfusate hexose concentrations and were greater for jejunal than for ileal loops when nonchloride solutions were infused. Net chloride absorption rates increased with infusate concentrations ranging from 0.2 per cent to 1.8 per cent NaCl, and were greater for ileal than for jejunal loops. However, this difference may be due to the greater chloride secretion of jejunal loops. Net fluid absorption rate decreased in curvilinear fashion with increasing perfusate osmolar concentration when NaCl or hexose was given. Net fluid absorption at given osmolar perfusate concentrations was greater from NaCl than from glucose or sorbose solutions. L.S.S.

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*Baldwin, Robert S.* (Marshfield Clin. Foundation, Marshfield, Wisc.): DBI (PHENFORMIN) IN THE TREATMENT OF DIABETES MELLITUS. Wisconsin M. J. *59*:359-62, June 1960.

In a group of sixty-five patients with whom phenformin was used for the control of diabetes, the results were considered excellent in twenty-seven, good in fifteen and poor in twenty-three. Nausea and vomiting are the only toxic effects thus far noted. This can be serious and in many instances necessitated discontinuance of the drug, even though the phenformin was effective in controlling the diabetes. The incidence of gastrointestinal upsets can be reduced by starting with small doses and increasing gradually. It appears that phenformin will find its greatest area of usefulness in three types of patients: the juvenile patients; the patients with whom other oral preparations have failed; and brittle diabetic patients in whom the use of a combination of phenformin and insulin can greatly reduce the incidence of hypoglycemic reactions. W.R.K.

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*Battaglia, Frederick C.; Meschia, Giacomo; Blechner, Jack; and Barron, Donald H.* (Dept. of Physiol., Yale Univ. School of Medicine, New Haven, Conn.): EFFECT OF INSULIN AND POISONS ON GLUCOSE UPTAKE OF SHEEP AND GOAT PLACENTA. Am. J. Physiol. *200*:64-66, January 1961.

Based upon the observation that in the ungulates, the sheep and the goats, the tissues of the placenta are divided into and can be obtained for study in three distinct parts, the authors have studied certain characteristics of these three placental components with respect to glucose uptake. The three components surveyed were (1) the chorion, derived entirely from the fetus; (2) the uterine mucosa of maternal origin; and (3) the cotyledons, composed of chorionic tissue which invades the uterine subepithelial tissue to become a structure with both fetal and maternal elements.

The basal glucose uptake of the cotyledons of sheep and goats was found to be 1.44 ± 0.09 mg./gm./hr.; that of the fetal chorion to be 2.22 ± 0.11; and that of the maternal uterine mucosa to be 2.05 ± 0.16 mg./gm. of tissue/hr. The tissue was obtained from pregnant animals between forty-five and fifty-five days' gestation. Insulin, at a concentration of 100 mU/ml., has no effect on net glucose utilization of any of these tissues. Nitrogen and cyanide both increased the basal uptake of cotyledons by approximately 70 per cent. Phlorizin was shown to inhibit both the basal uptake of glucose and the cyanide-accelerated uptake of glucose.

It is of interest that insulin does not affect the glucose utilization of any of the three tissues separating the fetus and the mother of the sheep and the goat, but these results do not exclude the possibility that insulin may exert an effect upon the transfer of monosaccharides from maternal to fetal extracellular fluid. L.S.S.

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*Beaser, Samuel B.* (Harvard Medical School, Abraham Rudy Diabetes Clin., Beth Israel Hosp., Boston, Mass.): ORALLY GIVEN COMBINATIONS OF DRUGS IN DIABETES MELLITUS THERAPY: FURTHER TRIAL. J.A.M.A. *174*:2137-41, Dec. 24, 1960.

A total of twenty-five ambulatory diabetic patients were treated with a combination of phenformin (DBI) and a sulfonylurea drug (either tolbutamide or chlorpropamide). These patients had failed to respond fully to treatment with a sulfonylurea drug alone. The therapy was successful in reducing blood sugar to satisfactory levels in nineteen patients, but three discontinued the treatment because of gastrointestinal intolerance. The therapy was unsuccessful in six patients, owing to the severity of the diabetes. W.R.K.

*Beckett, A. Gordon; and Lewis, J. G.* (Diabetic Dept., Royal Free Hosp., London, England): GOUT AND THE SERUM URIC ACID IN DIABETES MELLITUS. Quart. J. Med. *29*:443-58, July 1960.

It is concluded that diabetes increases the excretion of uric acid by a mechanism as yet unknown. Uric acid is considered to have a mild diabetogenic action. A family history of gout is present more frequently in patients with diabetes than in normal subjects. It is possible that a gene linkage exists between the two diseases. J.A.

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*Beigelman, Paul M.; and Onoprienko, Ilarion S.* (Dept. of Med., Univ. of Southern California Med. Sch. and the Los Angeles County Hosp., Los Angeles, Calif.): IMPROVEMENT OF ADIPOSE TISSUE INSULIN BIO-ASSAY BY LENGTHENING INCUBATION TIME. Metabolism *10*:162-64, February 1961.

Glucose uptake of rat epididymal fat was measured after two and six hours' incubation, with and without added insulin or normal human serum. The mean increase in glucose uptake in the presence of insulin was more significant at six hours than at two hours. Normal serum showed a significant insulin-like activity at six hours that was not apparent at two hours. Increasing the incubation period in this method of insulin assay may offer technical simplification and greater sensitivity, precision and reproducibility. C.A.R.

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*Bergen, Stanley S., Jr.; and Norton, William S., II* (St. Luke's Hosp., New York, N. Y.): 1-PHENETHYLBIGUANIDE—AN ORALLY ADMINISTERED HYPOGLYCEMIC AGENT. Postgrad. Med. *29*:194-98, February 1961.

This is a clinical study of the therapeutic effect of DBI in human diabetes. A total of fifty-four diabetic patients were studied: thirty on insulin and fourteen with unstable diabetes. Gastrointestinal side effects occurring up to three months after onset of therapy were noted in 33 per cent of patients, causing abandonment of therapy in a majority of them. No toxicity due to the drug was reported.

After six to twenty-four months, twenty-nine patients were still being treated with DBI, sixteen as the sole agent and thirteen with either insulin (for stabilization) or sulfonylurea drugs. In twenty-three of these the result was good regulation and in six, fair. The dosage range of the drug was 50 to 200 mg. per day (ave. 100 mg.) daily when used as the sole therapeutic agent and 25 to 100 mg. per day (ave. 60 mg.) when used in combination therapy. S.B.B.

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*Bergen, Stanley S., Jr.; and Van Itallie, Theodore B.* (Dept. of Med., St. Luke's Hosp., New York, N. Y.): THE GLUCAGON PROBLEM. N. Y. J. Med. *61*:779-86, March 1, 1961.

The authors review the current status of the glucagon problem. Glucagon is probably secreted by the alpha cells of the pancreas. Stimulation of hepatic glycogenolysis is its best defined action, although many extrahepatic effects have been reported in the last ten years. Relationship of glucagon secretion to the pituitary gland, the existence of a clinically manifest glucagon deficiency state, and its etiologic role in diabetes mellitus are problems that require further study. Clinically, glucagon has been used as a diagnostic test of liver function and in the treatment of various hypoglycemic states. C.A.R.

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*Bloom, Walter Lyon* (Research Lab., Piedmont Hosp., Atlanta, Ga.): CHANGES IN BLOOD KETONES DURING HEMORRHAGE AND SHOCK IN THE RAT. Metabolism *10*:171-73, February 1961.

Total blood ketones in unanesthetized Sprague Dawley rats rose significantly three to five hours after a 2.5-3.0 per cent blood loss. In three animals in the most severe shock, blood ketones did not exceed control levels in two and decreased in one. In these animals, unlike the others, blood amino acid nitrogen was elevated. Blood sugar was unaltered by blood loss except for hypoglycemia in the terminal phase of shock. These findings suggest the following course of events: As shock progresses the animal mobilizes energy sources; liver glycogen is depleted and blood sugar may eventually fall to hypoglycemic levels. Fat mobilization is manifested by ketonemia until hepatic metabolism is impaired by progressive shock, then ketone levels decline. At this stage, rising blood amino acid nitrogen may be an indication of failure of hepatic metabolism. C.A.R.

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*Chernosky, Marvin E.* (Dept. of Dermatology, Baylor Univ. Coll. of Med. and Hermann Hosp., Houston, Tex.): CURRENT CONCEPTS OF NECROBIOSIS LIPOIDICA. South M. J. *54*:25-29, January 1961.

Necrobiosis lipoidica is a histologically and clinically distinct cutaneous entity most frequently found on the anterior surface of the lower extremities in females. The lesions frequently present a problem for cosmetic reasons and because approximately one fourth of them will eventuate in ulcers. Whereas in the past it had been accepted that most of these patients either had or would eventually develop diabetes mellitus, recent reports and the statistical survey of 201 cases presented in this paper indicate that this is true in less than one half of the cases. The theories on the etiology and pathogenesis of the disease have been reviewed. A number of histologic findings have been reported in patients with necrobiosis lipoidica whether they have or do not have associated diabetes mellitus. Usually the control of the diabetes mellitus by hypoglycemic agents does not cause a disappearance of the skin lesions. The injection of hydrocortisone or prednisolone acetate into the lesion, preferably by use of the dermajector, causes complete disappearance of early lesions and improves to some degree the more advanced lesions. Apparently, also, it will stop the lesions from enlarging and progressing on to ulceration. The injection of heparin sodium solution into lesions has led to slight reduction of the yellowish color and histologic studies have shown a decrease in lipids in the treated tissue. Because early lesions respond best to injection of hydrocortisone or prednisolone, the physician should diagnose and recommend therapy early. Although less than one half of the cases of necrobiosis lipoidica are associated with diabetes mellitus, one should look for diabetes at intervals in patients who suffer from this cutaneous disease. W.R.K.

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*Cohen, A. M.* (Hebrew Univ. Hadassah Med. Sch., Jerusalem, Israel): PREVALENCE OF DIABETES AMONG DIFFERENT ETHNIC JEWISH GROUPS IN ISRAEL. Metabolism *10*:50-58, January 1961.

The prevalence of diabetes among Jews in Israel was studied by surveys of 15,961 persons (7,881 males and 8,080 females) during the years 1958-59. The Jewish community, for the purpose of this investigation, was divided into four ethnic groups: "Ashkenazim" (origin—Eastern and Western Europe, North America and South Africa); "Sephardim," (origin—the Mediterranean coast and Middle East); Yemenite new immigrants (ten years or less in Israel), and old or

"settled" immigrants (born in Israel or over twenty-five years' residence); Kurdish new immigrants, and old or settled immigrants (same definitions as for Yemenites). The age distribution in the communities studied was similar to that in official statistics. The prevalence of diabetes among the Ashkenazi group was 2.5 per cent (1.7 per cent in males, 3.0 per cent in females), and among the Sephardi group 1.0 per cent (0.8 per cent males, 1.1 per cent females). The difference between these two groups is not statistically significant. Diabetes among Yemenite new immigrants and Kurdish new immigrants was rare (0.06 per cent) but rose steeply in the communities of both groups after twenty-five years or more residence in Israel (for old or "settled" Yemenites 2.9 per cent and for old or "settled" Kurds 2.0 per cent). It is concluded that the prevalence of diabetes among Jews in Israel is not higher than among non-Jews in other countries and that environmental conditions are an important influence in determining appearance of the disease. C.A.R.

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*Cornblath, Marvin: Ganzon, Angelita F.: Nicolopoulos, Demetrios; Baens, Gloria S.;Hollander, Richard J.; Gordon, Mordecai H.; and Gordon, Harry H.* (Depts. of Pediat., Sinai Hosp. of Baltimore and Johns Hopkins Medical Institutions, Baltimore, Md.): STUDIES OF CARBOHYDRATE METABOLISM IN THE NEWBORN INFANT. III. SOME FACTORS INFLUENCING THE CAPILLARY BLOOD SUGAR AND THE RESPONSE TO GLUCAGON DURING THE FIRST HOURS OF LIFE. Pediatrics 27: 378-89, March 1961.

Sugars in capillary blood were measured during the first hours of life in full-term infants delivered vaginally and by cesarean section. Pretreatment with dextrose solution for mothers delivered vaginally did not affect the course of the blood sugar significantly. Pretreatment with dextrose or saline solution for mothers delivered by cesarean section produced significant differences in the course of the infants' blood sugar when compared to each other.

Full-term infants delivered vaginally who were more than six hours old had a greater hyperglycemic response to glucagon, 30 mg./kg., than infants less than three hours of age. Full-term infants delivered by elective cesarean section without previous labor responded to 30 mg./kg. of glucagon with a diminished hyperglycemia as compared to infants delivered vaginally. An exposure to labor influenced the neonate's response to glucagon whereas neither pretreatment of the mother nor the course of the blood sugar was associated with this response.

With large doses of glucagon, 300 mg./kg., the response differences associated with mode of delivery and maturity were not found to be significant. R.L.J.

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*Cummins, Leo H.* (Pediat. Sect., 97th Gen. Hosp., APO 757, U. S. Forces, New York, N. Y.): HYPOGLYCEMIA AND CONVULSIONS IN CHILDREN FOLLOWING ALCOHOL INGESTION. J. Pediat. 58:23-26, January 1961.

Two instances of hypoglycemia in children associated with ingestion of alcohol and producing convulsions are reported. One six-year-old boy was severely hypoglycemic for at least six hours. This child died with evidence of severe damage. The second was a three-year-old girl who made a complete recovery. Possible mechanisms are reviewed. In animals alcohol regularly produces hypoglycemia if the liver has been depleted of glycogen, which correlates with observations on alcoholic adults. The public health implication of these two cases, as regards accident prevention in children, are obvious. R.L.J.

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*Dembv, Allen M.* (Dept. of Ophthal., Bellevue Hosp., New York, N. Y.): DIABETIC RETINOPATHY AND RETINAL MICROANEURYSMS. J. Mt. Sinai Hosp. New York 28:53-61, January-February 1961.

The retinal microaneurysm is a feature of several diseases, each of which may be characterized to some extent by retinal anoxia (though they have not been reported to occur in many conditions in which anoxia is a prominent feature). Microaneurysms may represent physiologic efforts to restore retinal oxygenation, although the role of osmotic tension, recently publicized, may be a major factor in their pathogenesis. The relation of serum protein-bound carbohydrate, lipids and lipoproteins and adrenal hyperfunction to microaneurysms is unclear at this time. W.R.K.

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*Driscoll, Shirley G.: Benirschke, Kurt: and Curtis, George W.* (Depts. of Pathology and Obstet. and Gyn., Harvard Med. Sch., Boston Lying-in Hosp., Boston, Mass.): NEONATAL DEATHS AMONG INFANTS OF DIABETIC MOTHERS: POSTMORTEM FINDINGS IN NINETY-FIVE INFANTS. Am. J. Dis. Child. 100: 818-35, December 1960.

In spite of modern obstetric and medical management, the total fetal loss in pregnancies complicated by maternal diabetes mellitus is still appreciably higher than in nondiabetic women. The newborn infant of the diabetic mother shows anatomical and biochemical peculiarities which may be indicative of the mechanisms of fetal wastage operative under these conditions. The necropsy findings in ninety-five young infants of diabetic mothers have been reviewed. Fetal gigantism and generalized visceral enlargement were infrequent. As a group, these infants showed a tendency to cardiomegaly and to diminished weight of the brain. Hypertrophy and hyperplasia of the islets of Langerhans occurred in 81 per cent (forty-six of fifty-seven cases with suitable sections of pancreas). The degree of insular hyperplasia seemed to be correlated with relative body weight, being most marked in the heaviest infants at any gestational age. Adrenal enlargement was not impressive, but there was evidence of decreasd thymic-adrenal ratio. The hyaline membrane syndrome was the most frequent lethal process, accounting for 52 per cent (forty-nine) of the deaths. Fatal congenital malformations were relatively common, occurring in 17 per cent (sixteen) of the cases studied. These findings are discussed. The immediate problem is that of treatment or prevention of respiratory distress. Other stigmata of maternal diabetes in the newborn are manifestations of metabolic and hormonal interrelationships of mother and fetus. However, postmortem observations have limited application to problems of the living infant. W.R.K.

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*Epstein, Stephen E.: and Becker, E. Lovell* (New York Hosp.-Cornell Med. Center, New York, N. Y.): ACUTE GLOMERULONEPHRITIS IN AN ADULT WITH LONGSTANDING DIABETES MELLITUS. Ann. Int. Med. 54:97-103, January 1961.

This is a report of an adult diabetic patient with diabetes of long standing and acute glomerulonephritis. The diagnosis was made clinically by laboratory findings including percutaneous renal biopsy. The differential diagnosis between this condition and that of diabetic nephropathy was discussed. S.B.B.

*Field, James B.; and Johnson, Phyllis* (Clinical Endocrinology Branch, National Inst. of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.): EFFECT OF INSULIN ON D-XYLOSE DISAPPEARANCE IN DIABETIC PATIENTS. J. Appl. Physiol. *15*:979-82, November 1960.

The effect of intravenous insulin on the rate of disappearance of D-xylose has been studied in two nondiabetics and five diabetic patients. Although insulin accelerated the disappearance rate of D-xylose in nondiabetics, it had no effect on blood levels of D-xylose in insulin-treated diabetic patients. This inability of insulin to increase the rate of disappearance of D-xylose was not related to hyperglycemia since no effect was observed in a diabetic patient at a time when her blood sugar concentration was normal. Diabetes per se was not responsible for this lack of effect since insulin increased the rate of D-xylose disappearance in a diabetic patient who had never received insulin therapy. Since insulin caused a slower decline in the blood sugar of the insulin-treated diabetics than in the nondiabetics it is suggested that the rate at which insulin acts determines whether or not there will be an effect on D-xylose. In the insulin-treated diabetic, the presence of insulin antibodies could retard insulin action and account for the absence of an effect on D-xylose. J.A.

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*Fischer, Alfred E.* (Dept. of Pediat., Mount Sinai Hosp., New York, N.Y.): MANAGEMENT OF THE NEWBORN INFANT OF THE DIABETIC MOTHER. New York J. Med. *61*:292-96, Jan. 15, 1961.

An outline of the care of infants born to diabetic mothers is given. Mount Sinai Hospital has had a mortality rate of 7.6 per cent in the last 105 infants who have been tabulated. This figure is lower than that reported from many other clinics but is still far above that found in infants of normal mothers. Changes in the management of these infants have taken place over the years. There is hope for even better results in the future. W.R.K.

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*Freinkel, Norbert* (Thorndike Memorial Lab., 2nd/4th (Harvard) Med. Servs., Boston City Hosp., and the Dept. of Medicine, Harvard Medical School, Boston, Mass.): EXTRATHYROIDAL ACTIONS OF PITUITARY THYROTROPIN: EFFECTS ON THE CARBOHYDRATE, LIPID AND RESPIRATORY METABOLISM OF RAT ADIPOSE TISSUE. J. Clin. Invest. *40*:476-89, March 1961.

The effect of pituitary thyrotropic hormone (TSH) upon the metabolism of the epididymal adipose tissue of the rat was studied in vitro. The incubation was carried out at 38° for 90-180 minutes with labeled $C^{14}O_2$ and variable amounts of bovine albumin in the medium. The TSH was derived from beef and mouse pituitary tumors.

The TSH increased discharge of free fatty acids (FFA and NEFA) into the medium, increased oxygen consumption by the tissue (enhanced by increased amounts of glucose in the medium) and increased assimilation of glucose. The glucose oxidation was differentially greater for glucose-6-$C^{14}$ as compared with glucose-1-$C^{14}$.

The addition of oleate or palmitate to the medium resulted in both an increase in the intracellular pool of FFA as well as an increase of glucose oxidation, again differentially almost specific for glucose-6-$C^{14}$. It was concluded that the lipid effect was probably the primary action in the mechanism of these varied effects of TSH. S.B.B.

*Gardberg, Manuel* (New Orleans, La.): THE MANAGEMENT OF THE DIABETIC. J. Louisiana M. Soc. *112*:431-34, November 1960.

This paper attempts to direct attention to the human aspects of the management of the diabetic patient. It contains opinions derived from a large experience with diabetics who have been closely observed by one physician as they lived through a generation of time; some from infancy to parenthood, some from adolescence to middle life, some from middle life to an advanced age. The author stresses the psychological management of diabetes, especially that of children, so that the diabetic is not the victim of fears which alter unfavorably the whole outlook on life. W.R.K.

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*Gardiner, D. C.; Martin-Hernandez, D.; and Young, F. G.* (Dept. of Biochemistry, University of Cambridge, England): INFLUENCE OF OXYGENATION AND DEOXYGENATION ON DISTRIBUTION OF INSULIN ACTIVITY AMONG BLOOD-PLASMA-PROTEIN FRACTIONS. Lancet *2*:1269-71, Dec. 10, 1960.

The increase in insulin activity of the blood-plasma which results from oxygenation of the blood is associated with an alteration in the distribution of insulin activity among plasma-protein fractions. In particular, the insulin activity of the $\gamma$-globulin fraction is raised. The possibility is discussed that oxygenation of the blood causes a reversible depression in the activity of insulin inhibitors or antagonists present in plasma proteins, as well as a possible redistribution of them among the protein fractions. Other mechanisms clearly may play a part. Such a postulated depression in activity of insulin inhibitors or antagonists would be reversed when blood passes naturally from the arterial to the venous state, or is deoxygenated in vitro. Possibly the lungs not only oxygenate the blood passing through them but also alter the biological activity of some of the constituents of the blood plasma, of which insulin is one. J.A.

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*Gaunt, Robert; Chart, J. J.; and Renzi, A. A.* (Research Dept., Ciba Pharmaceutical Products, Inc., Summit, N.J.): ENDOCRINE PHARMACOLOGY. Science *133*:613-21, March 3, 1961.

The authors point out the present relatively undeveloped status of endocrine pharmacology, review the currently available drugs for the pharmacological regulation of the endocrine function including the islets of Langerhans, and foresee the future development of endocrine pharmacology along a wide front. L.S.S.

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*Goldstein, M. S.* (Dept. of Metabolic & Endocrine Res., Michael Reese Hosp., Chicago, Ill.): HUMORAL NATURE OF HYPOGLYCEMIA IN MUSCULAR EXERCISE. Am. J. Physiol. *200*:67-70, January 1961.

Muscular exercise leads to hypoglycemia. The "glucose transport theory" of insulin action places cellular entry of glucose as the limiting step in its rate of metabolism at rest as well as under exercise. Activation of entry of appropriate sugars by exercise, as a primary event, leads to the augmented rates of glucose metabolism seen with contraction of muscles. The mechanism of hypoglycemia of exercise is shown to be humoral in nature. Blood and lymphatic fluid of working dogs, both normal or diabetic, are shown to develop hypoglycemic properties during exercise which can be transferred to resting preparations. This hypoglycemic factor of muscle work is highly labile and appears to be quite distinct from insulin. L.S.S.

*Goodner, Charles J.; and Freinkel, Norbert* (Thorndike Memorial Lab., 2nd/4th (Harvard) Med. Servs., Boston City Hosp., Boston, Mass., and the Howard Hughes Med. Inst., Miami, Fla.): STUDIES OF ANTERIOR PITUITARY TISSUE IN VITRO: EFFECTS OF INSULIN AND EXPERIMENTAL DIABETES MELLITUS UPON CARBOHYDRATE METABOLISM. J. Clin. Invest. *40*:261-72, February 1961.

The glucose metabolism of the anterior pituitary gland was studied in vitro. The parameters of glucose assimilation studied were oxidative decarboxylation, glycogenesis, and lipogenesis. There was a 24 per cent and 16 per cent augmentation in such metabolism in the anterior pituitary tissues from the normal calf and rat respectively as a result of the addition of insulin. The increased production of $C^{14}O_2$ was defined as coming more from an increased activity of the Embden-Myerhof pathway than from the phosphogluconic pathway.

The carbohydrate assimilation was measurably decreased in the pituitary glands from diabetic (depancreatized or alloxanized) rats and this decrease was corrected by pretreatment with insulin. S.B.B.

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*Hugh-Jones, Kenneth; Newcomb, Alvah; and Hsia, David Yi-Yung* (Genetic Clin., Children's Memorial Hosp. and Dept. of Pediat., Northwestern University Medical School, Chicago, Ill.): THE GENETIC MECHANISM OF GALACTOSAEMIA. Arch. Dis. Childhood *35*:521-28, December 1960.

A family is presented in which the grandfather was proved to have galactosemia by galactose tolerance test and determination of blood levels of Gal-1-P uridyl transferase. His six children were proved by enzyme studies to be heterozygous carriers. The marriage of one of these to another heterozygote produced two affected children out of a total of four. Review of the literature suggests that galactosemia is transmitted as an autosomal recessive trait, and the study of this family yields data in support of this theory. R.L.J.

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*Ivy, A. C.; Setty, C. S. N.; and Suzuki, R.* (Dept. of Clinical Sci., Univ. of Illinois Coll. of Med., Chicago, Ill.): RELATION OF DIETARY CHOLESTEROL AND FAT TO ATHEROMATOSIS IN LAYING AND NONLAYING HENS. Am. J. Physiol. *200*:71-74, January 1961.

The authors report hens to be suitable subjects for the study of the relation of dietary cholesterol and fat to atheromatosis since chickens spontaneously develop macroscopic atheromatosis after five to six months of age. Further, the effect of hyperlipemia can be distinguished from that of hypercholesterolemia in hens fed similar diets as laying hens compared with nonlaying hens exhibit a three or fourfold increase in the total serum lipids with only a very slight elevation in the serum cholesterol.

Twenty-four female chicks received a diet containing 0.05 per cent cholesterol, 3 per cent fat and 22 per cent protein for six months. Then, twelve of them, six good layers and six non- or poor layers, received a diet containing 0.03 per cent cholesterol, 2.4 per cent fat and 14.25 per cent protein for fifty-two weeks. The other twelve, also divided into six good layers and six non- or poor layers, received a diet containing 0.08 per cent cholesterol, 7 per cent fat (6.5 gm./100 gm. of diet being beef tallow, m.p. 42° C.) and 17.44 per cent protein, the relative energy values being 774 and 1001 calories, respectively. These diets were fed twelve months, at the end of which period the chickens were eighteen months of age.

The chickens were then examined for the presence of atherosclerosis and its severity graded. In the twelve hens on the "low-fat, low-cholesterol" diet, the incidence was 25 per cent and the severity of atheromatosis was 5 per cent of the maximum possible severity; in the twelve on the "high-fat" diet it was 75 per cent and 25 per cent, respectively. There was no difference in the incidence and severity of atheromatosis between the "layers" and "nonlayers." Since egg laying is associated with a marked hyperlipemia and not a hypercholesterolemia, these results show also that a hyperlipemia alone though present for almost one year does not increase the rate of accumulation of cholesterol in the arteries of hens. L.S.S.

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*Jorgensen, Charles R.; Landau, Bernard R.; and Wilson, T. Hastings* (Dept. of Physiol. & Biological Chemistry, Harvard Medical School, Boston, Mass.): A COMMON PATHWAY FOR SUGAR TRANSPORT IN HAMSTER INTESTINE. Am. J. Physiol. *200*:111-16, January 1961.

The competition between different sugars for the transport mechanism of hamster small intestine was tested by an in vitro method which allowed the use of a single segment of intestine for both control and experimental periods. The transport of the test sugar D-galactose was inhibited by other sugars known to be actively absorbed by the intestine; namely, D-glucose, a-methyl-D-glucoside, 1-deoxy-D-glucose, 6-deoxy-D-glucose and 3-o-methyl-D-glucose. On the other hand D-mannose and D-xylose, two sugars not actively transported, did not inhibit D-galactose absorption. In addition, sugars known to be actively absorbed produced an inhibition of transport of D-glucose and 6-deoxy-D-glucose when these were selected as test sugars. The results of these experiments are consistent with the view that all transported sugars compete for a common pathway in hamster intestine. The structural requirements essential for this sugar transport are designated. The hypothesis is offered that transport of glucose across the cell permeability barrier is effected by ATP activation of a combination of glucose with a specific protein molecule in the cell membrane. L.S.S.

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*Kalant, N.; and Harland, W. Arthur* (Res. Lab. and Dept. of Pathology, Jewish Gen. Hosp., and the Dept. of Investigative Medicine, McGill University, Montreal, Canada): THE EFFECT OF AN ATHEROGENIC DIET ON NORMAL AND ALLOXAN-DIABETIC RATS. Canad. M. A. J. *84*:251-54, Feb. 4, 1961.

The atherogenic effects of a free diet for a period of 170-185 days was investigated in rats, thirty alloxan diabetic and twenty-one normal control animals. Large areas of myocardial damage were noted, ascribable to narrowing and thrombi of smaller coronary arteries, but not of any major vessels. Sudanophilic staining of the aorta was noted with the histological finding of fat within a superficial layer of macrophages in the intima. Statistically the difference in incidence of these lesions between the diabetic and nondiabetic animals was not significant but a larger group will be studied for this purpose.

Total serum protein, cholesterol and lipids were increased in all the animals. The latter two were significantly higher in the diabetic animals, with a specific elevation of the beta-lipoprotein level among the diabetic animals. S.B.B.

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*Kinch, Kenneth E.* (Imperial Oil Limited, Canada): DIABETES IN INDUSTRY—A REVIEW. M. Bull. *21*:63-84, March 1961.

Where routine health examinations are performed on a

regular basis, the turnover of the diabetic population should be fairly constant. It is apparent that diabetics fare better when diagnosed early, are referred early for treatment, and are kept under frequent observation. The majority of diabetic employees are capable of returning to their regular work. The exceptions are the employees taking insulin, the brittle diabetic and the diabetics who have developed severe complications.

The problem of obesity is vast and important both in precipitating the disease and in the treatment of it. In our own series 80.6 per cent of diabetics who were controlled had lost weight, 77.8 per cent of the uncontrolled had not lost weight or had gained. Information from the recent literature tends to add credence to the feeling that it does not matter whether an individual is an overt diabetic or a prediabetic as far as prognosis is concerned as the potential dangers are there. This has been particularly well shown in work done with prediabetic and diabetic pregnant women.

There appears to be a gradually changing concept about diabetes. Diabetes is no longer considered a simple metabolic illness characterized by a relative lack of insulin, which manifests itself by an elevated blood sugar. It would appear that physicians should not state glibly that they can prevent the onset of diabetes or prevent complications from occurring. At the most they may modify or delay the onset of the disease and its complications. W.R.K.

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*Laszlo, John; Harlan, William R.; Klein, Robert F.; Kirshner, Norman; Estes, E. Harvey, Jr.; and Bogdonoff, Morton D.* (Depts. of Medicine and Biochemistry, Duke University Med. Center and Durham Veterans Administration Hosp., Durham, N.C.): THE EFFECT OF 2-DEOXY-D-GLUCOSE INFUSIONS ON LIPID AND CARBOHYDRATE METABOLISM IN MAN. J. Clin. Invest. *40*:171-76, January 1961.

The action of 2-deoxy-D-glucose as a competitive inhibitor of glucose utilization is known. The authors attempted to study this effect by measuring the rise of free fatty acid (FFA) in the blood following its infusion into normal human subjects. The drug was administered intravenously over a thirty-minute period as a dose of 60 mg. per kilogram body weight in 100 cc. of normal saline. The blood glucose, free fatty acid and lactate all were studied for three or four hours. The plasma free fatty acid and glucose rose rapidly and simultaneously. The former rose to a peak of about three times the control value in forty-five minutes and then fell; the latter rose steadily for six to eight hours. It was calculated that this represented an average net glucose release of 40 gm. per hour. The subjects experienced drowsiness, hunger and sweating suggestive of hypoglycemia.

The rise in plasma FFA was reversed by insulin and glucose infusion but only the latter reversed the symptoms. There was an increased urinary excretion of catechol amines suggesting an adrenal medullary mechanism for the plasma FFA response in vivo. This drug does not have lipolytic properties when used in vitro. S.B.B.

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*Laufer, A.; and Stein, Olga* (Dept. of Pathology, Hebrew University—Hadassah Medical School and Hadassah University Hosp., Jerusalem, Israel): THE EXUDATIVE LESION IN DIABETIC GLOMERULOSCLEROSIS. Am. J. Clin. Path. *32*:56-61, July 1959.

The authors investigated eighteen diabetic and forty-seven nondiabetic cases with arteriosclerosis for the exudative form of glomerulosclerosis. The latter was found with greater frequency and intensity in diabetic patients. Adrenal cortical hyperplasia, severity of exudative glomerulosclerosis and the degree of arteriosclerosis were correlated. Clinical shock had occurred terminally more often in diabetic than in nondiabetic patients with exudative glomerulosclerosis. G.D.M.

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*Lazarus, Sydney S.; and Volk, Bruno W.* (Isaac Albert Res. Inst. of Jewish Chron. Dis. Hosp., Brooklyn; and Dept. Path., Albert Einstein Coll. of Med., New York, N.Y.): PANCREAS IN MATURITY-ONSET DIABETES: PATHOGENIC CONSIDERATIONS. Arch. Path. *71*:44-59, January 1961.

Pancreases of fifty maturity-onset diabetic patients and of fifty nondiabetic arteriosclerotic or hypertensive patients were studied histologically. Almost all the former showed abnormalities including arteriolosclerosis, arteriosclerosis, intrainsular pericapillary fibrosis, hyalinization of islets, perilobular and interacinar fibrosis and atrophy. The nondiabetic group showed similar but less severe and less frequent lesions.

Many of the pancreases from diabetic patients showed numerous well granulated beta cells. It was presumed that these cells were unresponsive to the normal stimulus of hyperglycemia because concomitant impairment of islet blood supply restricted the passage of glucose from the blood to the [illegible] cells. H.L.W.

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*Lie-Kian-Joe; Eng, Njo-Injo Tjoei; Tjokronegoro, Sutomo; Schaafma, Sj.; and Emmons, Chester W.* (University of Indonesia School of Medicine, Djakarta, Indonesia, and National Inst. of Allergy and Infec. Diseases, U.S. Dept. of Health, Education and Welfare, Bethesda, Md.): PHYCOMYCOSIS OF THE CENTRAL NERVOUS SYSTEM, ASSOCIATED WITH DIABETES MELLITUS IN INDONESIA. Am. J. Clin. Path. *32*:62-70, July 1959.

Case report of a fifteen-year-old girl with uncontrolled diabetes mellitus who had phycomycosis (mucormycosis) involving the brain via the nasal cavity and orbit. Twenty-three previously published cases are also reviewed. G.D.M.

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*Longfield, G. Maxwell; Holland, Doris E.; Lake, Anita J.; and Knights, Edwin M., Jr.* (Dept. of Path., Hurley Hosp., Flint 2, Mich.): COMPARISON STUDIES OF SIMPLIFIED TESTS FOR GLUCOSURIA AND PROTEINURIA. Am. J. Clin. Path. *33*:550-52, June 1960.

The combination dip-stick test for glycosuria and proteinuria (Uristix) was compared with the glucose reagent tablet test (Clinitest), the single dip-stick test (Albustix) and with Exton's sulfosalicylic acid test. The results of 2,700 urine specimens so examined compared favorably for the combination test. Significant discrepancies occurred in but 0.2 per cent of specimens on test for proteinuria and 1.8 per cent for glycosuria. G.D.M.

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*Mabry, C. Charlton; DiGeorge, Angelo M.; and Auerbach, Victor H.* (Depts. of Pediat. & Physiol. Chem., Temple University School of Medicine; St. Christopher's Hosp. for Children, Philadelphia, Pa.): LEUCINE-INDUCED HYPOGLYCEMIA. I. CLINICAL OBSERVATIONS AND DIAGNOSTIC CONSIDERATIONS. J. Pediat. *57*:526-38, October 1960.

Two infants with spontaneous hypoglycemia were found to be sensitive to leucine. A technic is described for carrying out an intravenous leucine tolerance test. A review of the literature suggests that approximately a third of infants with spontaneous hypoglycemia are sensitive to leucine. The diagnosis of this disorder and problems arising in its management are discussed. R.L.J.

*Mabry, C. Charlton; DiGeorge, Angelo M.; and Auerbach, Victor H.* (Depts of Pediat. & Physiol. Chem., Temple University, School of Medicine; St. Christopher's Hosp. for Children, Philadelphia, Pa.): LEUCINE-INDUCED HYPOGLYCEMIA. II. STUDIES CONCERNING OTHER AMINO ACIDS AND LEUCINE METABOLITES. J. Pediat. 57:539-47, October 1960.

Studies were carried out on control subjects and infants known to have leucine-sensitive hypoglycemia to test the effects on blood sugar of other L-amino acids, D-leucine, and some of the degradation products of L-leucine. None of the eighteen L-amino acids tested showed any significant, reproducible effect. D-leucine produced hypoglycemia three times out of the four times it was tested. It is suggested that the effect of D-leucine may be due to its conversion to L-leucine in the body. Isovaleric acid did not produce hypoglycemia in the leucine-sensitive subjects, contrary to previous reports. Alpha-ketoisocaproic acid produced responses similar to those induced by L-leucine in the affected patients except that the responses were less consistent. Excessive amounts of keto acids were not found in the urine, even after loading tests, suggesting that there is no defect in the conversion of alpha-ketoisocaproic acid to isovaleric acid. Increases in blood insulin and insulin activity were found in affected patients following leucine administration. Possible explanations of the metabolic derangement causing this disease are discussed.

R.L.J.

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*Marrack, David; Marks, Vincent; and Rose, F. Clifford* (National Hosp., Queen Square; King's College Hosp.; St. George's Hosp.; London, England): A LEUCINE-SENSITIVE INSULIN-SECRETING TUMOUR. Lancet 2:1329-30, Dec. 17, 1960.

Three patients with insulin secreting tumors were studied. Only one case showed a fall of blood glucose when fed L-leucine (0.1 gm. per kg.). After removal of the tumor no further clinical attacks of hypoglycemia were experienced and the patient did not respond to the oral leucine test repeated after operation. J.A.

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*Molnar, George D.; McGuckin, Warren F.; Striebel, James L.; and Gastineau, Clifford F.* (Mayo Clinic, Rochester, Minn.): EFFECTS OF THERAPEUTIC MEASURES IN A HYPERLABILE DIABETIC. Proc. Staff Meet. Mayo Clin. 36:45-61, Feb. 1, 1961.

The authors report metabolic studies of 142 days' close observation of a hyperlabile diabetic patient. Three or more daily doses of short-acting insulin gave statistically and clinically better diabetic regulation than single daily injections of longer acting insulin although lability was not eliminated completely by the multiple dose regimen. Administration of DBI in conjunction with a single daily dose of insulin did not improve regulation or enhance stability. Changes in dietary composition did not conclusively modify lability or regulation. There was no detectable effect of exercise on arterial or venous blood glucose levels regardless of whether or not insulin was administered. Insulin sensitivity in this patient was confirmed and emphasized the importance of accurate timing of insulin dosage in relation to meals. C.A.R.

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*Naide, Meyer; and Schnall, Charles* (Dept. of Med., Women's Med. Coll. of Pennsylvania, and Dept. of Med., Albert Einstein Med. Center, Philadelphia, Pa.): BONE CHANGES IN NECROSIS IN DIABETES MELLITUS: DIFFERENTIATION OF NEUROPATHIC FROM ISCHEMIC NECROSIS. Arch. Int. Med. 107:380-83, March 1961.

The X ray is of prime importance in differentiating ischemic from neuropathic lesions in the feet of diabetic patients. Paradoxically, the prognosis of a foot with soft-tissue necrosis is much better if bone destruction or resorption is present on X ray indicating neuropathy, than if soft-tissue necrosis is present in an ischemic foot with the bone intact. The explanation for the better prognosis in the patient with the neuropathic bone lesion is the fact that in order for rarefaction, lysis, or destruction of bone to occur there must be blood flowing through it. If blood supply is severely reduced bone will not undergo destruction or lysis. W.R.K.

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*Osler, Mogens; and Pedersen, Jørgen* (Royal Maternity Dept. B, Rigshospitalet, Copenhagen, Denmark): THE BODY COMPOSITION OF NEWBORN INFANTS OF DIABETIC MOTHERS. Pediatrics 26:985-92, December 1960.

Newborn infants of diabetic mothers show many deviations from normal, among which a high perinatal mortality and a high birth weight are most characteristic. Studies on the body composition of newborn infants of diabetic mothers (diabetic infants) are reported. Birth weight and length, total body water, water compartments, body fat, osseous maturity, and urinary excretion of water, electrolytes and nitrogen were determined in newborn infants of diabetic mothers, and for comparison in normal full-term infants (normal infants) and newborn infants of the same gestational age as the first group, but born to nondiabetic mothers (control infants). Infants of diabetic mothers must be characterized as infants whose maturity in terms of development of ossification centers, excretion of water, electrolytes and nitrogen, and other organ functions corresponds to their chronologic age, or even less. Besides, they are grossly overweight on account of a surplus of fat and to a lesser degree of carbohydrate stores in tissues and organs. The combination of premature organ functions, increased fat content and water release on account of breakdown of protein and carbohydrate stores after birth may be major factors in the neonatal pulmonary syndrome exhibited by infants of diabetic mothers. Discussing the influence of glucose plus insulin, growth hormone and adrenal cortical steroids upon the body composition, the authors conclude that the changes found in newborn infants of diabetic mothers may be due primarily to maternal hyperglycemia. W.R.K.

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*Owen, John A., Jr.* (Veterans Administration Hospital and Dept. of Internal Medicine, George Washington University, Washington, D.C.): EFFECTS OF HORMONE THERAPY IN PATIENTS WITH DIABETES. Virginia M. Month. 87:681-86, December 1960.

While endocrinopathy or exogenous hormone therapy may affect a coexisting diabetes, for practical purposes this effect is of little significance except for the use of adrenocortical steroids in the management of inflammatory or neoplastic disease. The effect of such steroids is to inhibit protein anabolism with a concomitant increased gluconeogenesis and glycogenolysis, leading to an increased hepatic release of glucose. Because of impaired fat synthesis and diversion of Krebs cycle intermediates, ketogenesis is also increased in the absence of insulin. Numerous powerful synthetic analogues of hydrocortisone are currently available and their diabetogenic potency appears to parallel their anti-inflammatory potency. Diabetic patients should not receive such steroids unless there is a serious threat to life and health which will respond to steroids and to nothing else. If such treatment is instituted,

the patient should be watched carefully with the physician ever ready to increase the dose of insulin as soon as there is any evidence of deterioration of diabetes control. This will suffice to control 99 per cent of such patients. If this fails, insulin may be supplemented by the addition of DBI, anabolic steroids or salicylates. If these measures, relatively untried at present, are unsuccessful, the dose of steroids must be reduced until satisfactory diabetic control is achieved. Future large-scale use of thyroxine analogues, growth hormones, and glucagon will raise new problems for the diabetic patient and his doctor. A thorough knowledge of endocrine physiology and diabetic biochemistry will continue to be essential to the proper use of hormones in clinical diabetes. W.R.K.

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*Petrelli, F.; Sartorelli, L.; and Siliprandi, N.* (University of Padova, Italy): THE ACTIGN OF GLUCOSE-1-PHOSPHATE ON THE GLYCOGEN STORES OF NORMAL AND DIABETIC ANIMALS. Italian J. Biochem. 9:50-53, January-February 1960.

Glucose-1-phosphate is the metabolite more closely related to glycogen through the phosphorolysis reaction under the influence of phosphorylase. It was thus of interest to see if the administration of glucose-1-phosphate is able to modify the glycogen stores to a greater extent than glucose which, as is known, before forming glycogen, must first be transformed into glucose-1-phosphate. The administration of glucose-1-phosphate to normal animals modified the liver and muscle glycogen stores to the same extent as the administration of an equivalent quantity of glucose. On the other hand, glucose-1-phosphate produced in diabetic muscle an increase in glycogen which was appreciably greater than that determined by glucose. In the diabetic animal, the administration of glucose and glucose-1-phosphate produced the same change in liver glycogen. W.R.K.

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*Pines, Kermit L.* (Dept. of Med., Presbyterian Hosp., New York, N.Y.): DIABETIC ACIDOSIS. New York J. Med. 61: 925-31, March 15, 1961.

The author reviews and discusses current hypotheses regarding the pathogenesis of diabetic acidosis. The clinical picture is also described, and the treatment used at the Presbyterian Hospital in New York City is presented. C.A.R.

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*Rafnelsen, Ole J.* (Dept. of Neurol., Copenhagen Univ. Sch. of Med., Copenhagen, Denmark): STUDIES ON A DIRECT EFFECT OF INSULIN ON THE CENTRAL NERVOUS SYSTEM: A REVIEW. Metabolism 10:99-114, February 1961.

The central nervous system has been generally considered to belong to the group of tissues on which insulin exerts no direct effects. However, in recent experiments it has been shown that insulin increases the glucose uptake of tissue samples from the central nervous system that are at least in part covered with natural membranes, e.g., rat spinal cord, "first" brain slices and cerebellum. However, synthesis of glycogen, conversion of glucose to lactate or carbon dioxide, or glucose incorporation into water-soluble glycolipids was not increased. The scanty literature dealing with a possible direct effect of insulin on the central nervous system is reviewed. The results obtained by different workers are contradictory, but it can be concluded that insulin may have a direct effect on the central nervous system. C.A.R.

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*Schwarz, V.* (Depts. of Child Health and Pathology, Univ. of Manchester, England): THE VALUE OF GALACTOSE PHOSPHATE DETERMINATIONS IN THE TREATMENT OF GALACTOSAEMIA. Arch. Dis. Childhood 35:428-32, October 1960.

A technic is described for measuring the concentration of galactose-1-phosphate levels in erythrocytes. Assuming that the manifestations of this disease are due to accumulation of Gal-1-P in the cells, it is suggested that this test is a good index of the adequacy of treatment, since it is the author's feeling that disappearance of symptoms is an inadequate guide. R.L.J.

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*Sidbury, James B., Jr.; Cornblath, Marvin; Fisher, Jean; and House, Evelyn* (Dept. of Pediat., Johns Hopkins Univ. School of Medicine, Baltimore, Md.): GLYCOGEN IN ERYTHROCYTES OF PATIENTS WITH GLYCOGEN STORAGE DISEASE. Pediatrics 27:103-11, January 1961.

The authors discuss the various types of glycogen storage disease from the standpoint of the enzymatic abnormalities present and types and location of glycogen stored. A technic is described for measuring glycogen concentration in erythrocytes and white blood cells. Enzymatic degradation and iodine studies were used to characterize the glycogen obtained. Erythrocyte glycogen was found to be elevated in patients with type III glycogen storage disease (due to a deficiency of the debrancher enzyme, amylo-1, 6-glucosidase). One patient with type IV disease (amylo-1, 4-1, 6-transglucosidase deficiency) was found to have the typical abnormal glycogen in his erythrocytes and white cells. These procedures are said to yield an etiologic diagnosis in about 50 per cent of patients with glycogenosis, and avoid the dangers of liver biopsy in these patients, many of whom have a bleeding tendency, hypoglycemia, and a tendency to ketosis and acidosis. R.L.J.

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*Sokal, Joseph E.; Lowe, Charles U.; Sarcione, Edward J.; Mosovich, Luis L.; and Doray, Bernard H.* (Div. of Medicine, Roswell Park Memorial Inst. and the Statler Res. Labs. of the Children's Hosp. and the Dept. of Pediat., The University of Buffalo School of Medicine, Buffalo, N.Y.): STUDIES OF GLYCOGEN METABOLISM IN LIVER GLYCOGEN DISEASE (VON GIERKE'S DISEASE): SIX CASES WITH SIMILAR METABOLIC ABNORMALITIES AND RESPONSES TO GLUCAGON. J. Clin. Invest. 40:364-74, February 1961.

The authors studied the glycogen metabolism in vitro in liver and muscle biopsies from children with von Gierke's disease. The decreased breakdown of hepatic glycogen of liver was accompanied by an anomalous conversion of glycogen to lactate under the influence of glucagon.

In vivo, high blood lactate values were observed. These blood lactate levels were further increased after intravenous infusions with glucagon while the blood glucose level rose little. The reverse effects occurred after glucagon in normal children. The blood lactate levels were enhanced by fasting and decreased by feeding and especially by glucose infusions.

The authors concluded that in this disease the principal end product of liver glycogen breakdown was not glucose but lactic acid both spontaneously or under the influence of glucagon. S.B.B.

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*Taylor, C. Bruce; Cox, George E.; and Trueheart, Richard E.* (Northwestern Univ. Sch. of Med., Chicago, and Evanston Hosp., Evanston, Ill.): REVERSIBILITY OF ATHEROSCLEROSIS. Illinois M. J. 119:80-81, February 1961.

The evidence for reversibility of atherosclerosis includes: (1) disappearance of atheromatous lesions in experimental animals after reduction of serum cholesterol; (2) diminution and disappearance of skin and tendon xanthomas in patients

following fall in serum cholesterol; (3) less than expected atherosclerosis at autopsy in patients dying after long periods of dietary restriction and weight loss.

Atherosclerotic plaques produce their harmful effects by impinging on the vessel lumen; by "foreign body" irritation of the vessel wall; and by the promotion of intramural abnormal vascularization which is subject to rupture with the formation of obstructing hematomas.

Clinical and experimental evidence suggests that lipids move into arterial tissue when serum cholesterol is above 250 mg. per cent and are resorbed from arteries at levels below 200 mg. per cent. H.L.W.

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*Unger, Roger H.; Madison, Leonard L.; and Carter, Norman W.* (University of Texas Southwestern Medical School, Dallas, Tex.): THE PROPER CHOICE OF ORAL ANTIHYPERGLYCEMIC THERAPY IN DIABETES MELLITUS. Postgrad. Med. 29: 40-45, January 1961.

The authors discuss the clinical use of oral antihyperglycemic drugs now available in the United States. They classify diabetics into "ketoacidosis-resistant" (maturity onset) and "ketoacidosis-prone" (growth onset) types, and indicate that oral antihyperglycemic drugs should never be used to replace insulin in the latter. They estimate that 70 per cent of all patients who fail to be regulated by tolbutamide and 60 per cent of all those failing with phenformin may successfully be treated by means of tolbutamide-phenformin combination therapy. They also estimate that this combination may effect diabetic control in 90 per cent of all ketoacidosis-resistant diabetic patients. S.B.B.

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*Unger, Roger H.; Madison, Leonard L.; and Carter, Norman W.* (Depts. of Medicine, Veterans Administration Hosp., University of Texas Southwestern Med. Sch., and Metabolic Clin., Parkland Memorial Hosp., Dallas, Tex.): TOLBUTAMIDE-PHENFORMIN IN KETOACIDOSIS-RESISTANT PATIENTS. J.A.M.A. 174:2132-36, Dec. 24, 1960.

Combined tolbutamide-phenformin therapy was evaluated in ketoacidosis-resistant diabetics imperfectly controlled by tolbutamide alone. In seventy patients in whom valid comparison with prior tolbutamide therapy was possible, significant reduction in the mean fasting blood sugar (FBS) level was accomplished in 70 per cent after a change from tolbutamide alone to combination therapy. The antihyperglycemic effectiveness of this regimen in the tolbutamide-hyporesponsive and unresponsive groups appears to exceed by many times that of chlorpropamide, metahexamide, and phenformin alone. Tolbutamide is the drug of choice in tolbutamide-responsive diabetes; tolbutamide-phenformin combination therapy appears to be an effective and relatively safe method of treating tolbutamide-hyporesponsive and unresponsive ketoacidosis-resistant diabetes. W.R.K.

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*Volk, Bruno W.; Saifer, Abraham; and Lazarus, Sydney S.* (Isaac Albert Res. Inst. of the Jewish Chronic Disease Hosp., Brooklyn, N.Y.): BLOOD SACCHAROIDS IN DIABETES MELLITUS. J. Lab. & Clin. Med. 57:367-76, March 1961.

Blood saccharoid levels of twenty nondiabetic subjects (eighty samples) and of thirty-five diabetic patients (140 samples) were determined as the difference between copper reduction and glucose oxidase methods. Mean values plus or minus the standard deviations for the normal and diabetic groups were 6.0 mg. per cent ± 4.0 and 21.0 mg. per cent ± 10.6, respectively. No significant difference was found between capillary and venous blood for either group. No changes were observed in the saccharoid levels of either diabetic or nondiabetic individuals during the modified glucose-insulin tolerance test or during the four-hour interval after a single oral dose of 1 gm. of tolbutamide. Elevation of the saccharoid level of the diabetic patients was not related to blood glucose values or to insulin sensitivity. By means of paper chromatographic technics an organic acid was found in increased amounts in barium-zinc filtrates from diabetic blood which had the same RF value as glucuronic acid. It is suggested that in some diabetic patients alternate pathways of carbohydrate metabolism might result in increased production of glucuronic acid or other saccharoids. Furthermore, the use of saccharoid levels as an index of metabolic control of diabetes is considered. W.R.K.

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*Weekly Clinical Pathological Exercises, Benjamin Castleman, M.D., Editor* (Massachusetts General Hospital, Boston, Mass.): PRESENTATION OF CASE. The New Eng. J. Med. 264:348-54, Feb. 16, 1961.

This is a case report involving a seventy-nine-year-old woman with onset of diabetes in middle life which became a labile type. She entered the hospital with weakness, lethargy, anorexia and mental confusion and evidence of diabetic acidosis. With insulin therapy and intravenous fluids, the blood sugar fell and there was some improvement although the patient continued to be drowsy and slightly stuporous. The terminal episode was associated with an acute renal failure. Pathological findings included diabetic glomerulosclerosis, a massive pulmonary embolus, evidence of coronary artery disease with myocardial infarctions, recent and old, phlebothromboses of the left superficial femoral vein and a massive pulmonary embolus, generalized arteriosclerosis, chronic pyelonephritis, lipomatosis of the pancreas, severe fatty liver, and ulcerative esophagitis and gastrointestinal hemorrhage. The pathologist commented that this case provides what might be called a museum of pathologic changes related to diabetes mellitus. The liver was of normal size but with large globules of fat occupying almost every liver cell, a change that is a frequent accompaniment of uncontrolled diabetes. The kidney was the site of a variety of changes, most striking finding being a classic diabetic glomerulosclerosis. Histologic study showed both diffuse and nodular hyaline changes in the glomeruli with marked hyaline deposition in the walls of the arterioles. In addition the patient had marked chronic pyelonephritis. There was extensive arteriosclerosis in most of the major vessels; examination of the brain was not permitted but the internal carotid artery showed extensive arteriosclerosis. S.S.

## *ORGANIZATION SECTION*

### OFFICERS AND MEMBERS OF COUNCIL, AMERICAN DIABETES ASSOCIATION, 1960-1961

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# 21st Annual Meeting
# American Diabetes Association
# June 24-25, Hotel Commodore, New York City

Rachmiel Levine, M.D., will deliver the Banting Memorial Lecture during the Scientific Session Saturday morning, June 24. His subject will be "Concerning Mechanisms of Insulin Action." Dr. Levine is Chairman and Professor, Department of Medicine, New York Medical College; and Director, Medical Services, Flower and Metropolitan Hospitals, New York.

### BANQUET HIGHLIGHTS

- Franklin B. Peck, Sr., M.D., Indianapolis, will preside at the Annual Banquet of the Association on Saturday evening, June 24.
- The Banting Medal will be presented to Dr. Levine.
- Deaconess Maude Behrman, Atlantic City, will offer the Invocation.
- The 1961 Lilly Award will be presented to Rosalyn S. Yalow, Ph.D., Assistant Chief, Radioisotope Service, Veterans Administration Hospital, Bronx, New York. Dr. Yalow has been instrumental in developing a sensitive immunoassay technic for determination of insulin in biological materials. Dr. Yalow will also present a paper entitled "Immunoassay of Insulin" during the Scientific Session Saturday afternoon, June 24.
- The Annual Citation as an Outstanding Layman will be awarded to Mrs. Carolyn Spiegel of the Chicago Diabetes Association by Maurice Protas, M.D., Chairman, Assembly of Delegates, American Diabetes Association.
- Ralph West Robey, Ph.D., will speak on "The Washington Scene and Money." He is currently Economic Adviser, National Association of Manufacturers, and formerly Financial Editor of *Newsweek* magazine.

### SCIENTIFIC SESSIONS

Five Scientific Sessions have been scheduled this year. These will be held as follows: Saturday, June 24, morning and afternoon; Sunday, June 25, two concurrent morning sessions and one in the afternoon. Thirty-seven papers will be presented and twenty-eight others will be included in the program by title.

Jerome W. Conn, M.D., is Chairman of the Committee on Scientific Programs. Members of the Committee include Blair Holcomb, M.D., Vice Chairman; James Ashmore, Ph.D.; Thaddeus S. Danowski, M.D.; Irving Graef, M.D.; and Harvey C. Knowles, Jr., M.D.

Although a joint meeting will not be held with The Endocrine Society this year, arrangements have been made so that Endocrine Society members may attend the Scientific Sessions of the ADA, and ADA members may attend, without charge, sessions on diabetes of The Endocrine Society at the Hotel Biltmore.

## REGISTRATION

Friday, June 23 (10:30 a.m.—6:30 p.m.), Lobby Floor; Saturday, June 24 and Sunday, June 25 (8:30 a.m.—5:00 p.m.) in the Windsor Gallery, Lobby Floor.

There is no registration fee for members of the American Diabetes Association or The Endocrine Society. Fee for nonmembers is $2.00, which includes a copy of the program.

## OTHER EVENTS

Thirty-three sessions of committees, boards and other groups are scheduled. A complete list of committee and other organizational meetings will appear in the printed program.

- The Annual Business Meeting will be held in the Windsor Ballroom, on the Lobby Floor of the Hotel Commodore on Sunday, June 25, from 2:00 to 2:45 p.m., at which Dr. Peck will give the Address of the President.
- A Social Hour in the Windsor Terrace at 6:30 p.m. will immediately precede the Banquet in the Windsor Ballroom, Saturday, June 24.

## THE PRINTED PROGRAM

During May a copy of the ADA program will be sent to all members at no charge. It will contain abstracts of papers to be presented as well as those included by title. Copies will also be available at the registration desk for $1.00 each.

Papers to be delivered at the Scientific Sessions are listed in the following program in the order of their presentation. Those given by title are also listed.

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## SCIENTIFIC SESSIONS

*Saturday morning, June 24, 9:00 a.m.*

FRANKLIN B. PECK, Sr., Chairman

### *Islets of Langerhans*

**Demonstration and Quantitation of Extractable Glucagon in Human Diabetic and Nondiabetic Pancreas**

ROGER H. UNGER, KATHERINE SIMS (by invitation), ANNA M. EISENTRAUT (by invitation), MARY S. McCALL (by invitation), and LEONARD L. MADISON, Veterans Administration Hospital and University of Texas Southwestern Medical School, Dallas, Tex.

**Experimentally-induced Sensitivity to Leucine Hypoglycemia in Healthy People and in Diabetic Subjects**

STEFAN S. FAJANS, RALPH F. KNOPF (by invitation), LAWRENCE POWER (by invitation), and JEROME W. CONN, University Hospital, Ann Arbor, Mich.

**Exhaustion of Insulogenic Reserve in Maturity-Onset Diabetics During Prolonged and Continuous Hyperglycemic Stress**

HOLBROOKE S. SELTZER, Veterans Administration Hospital and University of Texas Southwestern Medical School, Dallas, Tex.

**Ultrastructural Changes in Islets of the Rat Produced by Tolbutamide**

JOSEPH R. WILLIAMSON (by invitation), PAUL E. LACY, and JOE W. GRISHAM (by invitation), Washington University, St. Louis, Mo.

### *Diabetes and the Eye*

**Glucose Metabolism of the Normal Retina. Study of Glucose Uptake, Factors Influencing it and a Note Upon a Plasma Inhibitory Factor**

HARRY KEEN (by invitation), National Institutes of Health, Bethesda, Md., introduced by James B. Field.

**The Pathogenesis of Diabetic Retinal Microaneurysms**

J. M. B. BLOODWORTH, Jr., Ohio State University, Columbus, Ohio

**Corneal and Lenticular Opacities in the Eyes of Rats Following Long-term Administration of Sulfonylurea Derivatives**

HAROLD N. WRIGHT (by invitation), University of Minnesota Medical School, Minneapolis, Minn., introduced by Arnold Lazarow.

## BANTING MEMORIAL LECTURE

**Concerning Mechanisms of Insulin Action**

RACHMIEL LEVINE, Chairman and Professor, Department of Medicine, New York Medical College; Director, Medical Services, Flower and Metropolitan Hospitals, New York, N.Y.

*Saturday afternoon, June 24, 2:00 p.m.*

FRANKLIN B. PECK, Sr., Chairman

### *Hepatic Production and Utilization of Glucose*

**Endogenous Glucose Formation from C-14-labeled Precursors after Glucose Injection in Patients**

R. C. De MEUTTER and W. W. SHREEVE, Brookhaven National Laboratory, Upton, N.Y.

**Effects of Insulin on Glucose Production, Utilization, Accumulation Rates and "Space" in Dogs Determined by a Validated Tracer Method**

G. HETENYI (by invitation), G. A. WRENSHALL and CHARLES H. BEST, University of Toronto, Toronto, Ontario, Canada

**The Significance of the Liver in the Quantitative Disposition of Glucose Loads**

F. R. LECOCQ (by invitation), D. M. MEBANE (by invitation), B. COMBES and LEONARD L. MADISON, University of Texas Southwestern Medical School, Dallas, Tex.

### *Glucose Utilization in Muscle*

**Effect of Growth Hormone on Glucose Transport in Skeletal Muscle**

FRANK A. RIDDICK, Jr. (by invitation), DAVID REISLER (by invitation), and DAVID M. KIPNIS (by invitation), Washington University School of Medicine, St. Louis, Mo.

**Studies Concerning a Possible Humoral Factor Produced by Working Muscles which Influences Glucose Utilization**

WILLIAM E. DULIN and J. J. CLARK (by invitation), Research Laboratories, The Upjohn Company, Kalamazoo, Mich.

### *Insulin Resistance*

**Immunoassay of Insulin**

ROSALYN S. YALOW (by invitation) and SOLOMON A. BERSON (by invitation), Radioisotope Service, Veterans Administration Hospital, Bronx, N.Y.

**Studies on Steroid Treatment of Chronic Insulin Resistance**

JAMES B. FIELD, National Institutes of Health, Bethesda, Md.

**Insulin Resistant Diabetics**

JAMES C. BARRETT (by invitation), and BURIS R. BOSHELL, Medical College of Alabama, Birmingham, Ala.

*Sunday morning, June 25, 9:00 a.m.*

HOLBROOKE S. SELTZER, Chairman

*Insulin-like Activity and Modifying Influences*

**A Study of the Effects of Albumin from Normal and Diabetic Plasma upon Glucose Metabolism by the Rat Hemidiaphragm**

HARRY KEEN (by invitation), National Institutes of Health, Bethesda, Md., introduced by James B. Field.

**Serum Insulin-like Activity in Untreated Diabetes Mellitus**

PAUL M. BEIGELMAN, ROBERT E. TRANQUADA, ILARION S. ONOPRIENKO (by invitation), and ALBERT B. BENDER (by invitation), University of California Medical School and Los Angeles County Hospital, Los Angeles, Calif.

**Insulin-binding Antibodies in Plasma After Administration of Insulin to Rabbits**

JAMES L. STRIEBEL (by invitation), GEORGE D. MOLNAR and CHARLES A. OWEN, Jr. (by invitation), Mayo Clinic and Mayo Foundation, Rochester, Minn.

**Studies of Insulin Transport in Sera of Normal and Diabetic Patients**

VASANT V. ODAK (by invitation), THADDEUS E. PROUT and GEORGE J. DENDRINOS (by invitation), The Johns Hopkins University School of Medicine, Baltimore, Md.

*Triglyceride Metabolism in Diabetic Patients*

**Further Studies of the Defect in Triglyceride Synthesis in the Stable Adult Diabetic**

ALLEN R. HENNES, MARGARET Z. MOORE (by invitation), and SHAFEEK S. SANBAR (by invitation), Radioisotope Service, Veterans Administration Hospital, Oklahoma City, Okla.

**The Hyperlipemic Effect of a Low Fat, High Carbohydrate Diet in Diabetic Subjects**

EDWIN L. BIERMAN and JAMES T. HAMLIN, III (by invitation), The Rockefeller Institute, New York, N.Y.

**Some Aspects of Fatty Acid and Glyceride Metabolism in Normal and Diabetic Subjects**

LAURANCE W. KINSELL, GEORGE D. MICHAELS (by invitation), ROBERT E. VISINTINE (by invitation), and GEORGE FUKAYAMA (by invitation), Institute for Metabolic Research, Highland-Alameda County Hospital, Oakland, Calif.

*Sunday morning, June 25, 9:00 a.m.*

STEFAN S. FAJANS, Chairman

*Earlier Detection of the Manifestations of Diabetes*

**The Detection of Early Vascular Abnormalities in Diabetic Children**

HENRY LAX (by invitation), and ARTHUR W. FEINBERG (by invitation), Columbia University College of Physicians and Surgeons, New York, N.Y., introduced by Martin G. Goldner.

**Hyperlipemia as an Early Manifestation of Diabetes Mellitus**

ROBERT F. BRADLEY, R. ECCLES (by invitation), and A. E. FREEDLENDER (by invitation), Joslin Clinic, Harvard School of Public Health and Diabetes Foundation, Inc., Boston, Mass.

**Glucose Tolerance and Cortisone-glucose Tolerance in Juvenile Relatives of Diabetic Patients**

A. A. PUPO (by invitation), M. M. MACHADO (by invitation), BERNARDO L. WAJCHENBERG and EMILIO MATTAR (by invitation), Hospital das Clinicas and University of São Paulo Medical School, São Paulo, Brazil

**A Comparison of Several Simplified Glucose Tolerance Tests**

KELLY M. WEST, A. A. KYRIAKOPOULOS (by invitation), and J. A. WULFF (by invitation), University of Oklahoma Medical Center, Oklahoma City, Okla.

**Response of the Hypoglycemia of Mild Diabetes and Prediabetes to Tolbutamide**

PETER G. ECONOMOU (by invitation), WILL G. RYAN (by invitation), and THEODORE B. SCHWARTZ, Presbyterian-St. Luke's Hospital and the University of Illinois, College of Medicine, Chicago, Ill.

**Heredity and Diabetes**

IBRAHIM ZEYTIN, MERVYN LAKIN, PRISCILLA WHITE and OLIVE M. LOMBARD (by invitation), Joslin Clinic, Boston, Mass.

**Diabetic Nephropathy Occurring Without Manifest Diabetes**

MAX ELLENBERG, The Mount Sinai Hospital, New York, N.Y.

**The Insulin-binding Capacity of the Glomerular Nodules in Diabetic Nephropathy, as Demonstrated by Fluorescence Microscopy**

ALINE W. BERNS (by invitation), CECILIA T. OWEN (by invitation), and HERMAN T. BLUMENTHAL (by invitation), The Jewish Hospital, St. Louis, Mo.

*Sunday afternoon, June 25, 2:45 p.m.*

BLAIR HOLCOMB, Chairman

*Pregnancy, Birth Weight and Diabetes*

**Birth Weight and Diabetes**

IBRAHIM ZEYTIN, MERVYN LAKIN, OLIVE M. LOMBARD (by invitation), and PRISCILLA WHITE, Joslin Clinic, Boston, Mass.

**Urinary Estrogens in Diabetic Pregnancy —A Clinical Correlation**

G. E. JORON, R. HOBKIRK (by invitation), P. R. BLAHEY (by invitation), J. H. ROUTLEDGE (by invitation), and A. F. FOWLER, The Montreal General Hospital and McGill University, Montreal, Quebec, Canada

**Control of Perinatal Fetal Mortality in Pregnancy Complicated by Diabetes Mellitus**

ANTONIO L. COURT (by invitation), PALMER H. FUTCHER and W. NEWTON LONG (by invitation), The Johns Hopkins University School of Medicine and The Johns Hopkins Hospital, Baltimore, Md.

*Various Clinical Observations*

**Diabetes Mellitus in Guam**

JOSEPH KOVACS (by invitation), Medical Service of the Government of Guam, Agana, Guam, introduced by Jerome W. Conn.

**A Study of Diabetes Mellitus in Werner's Syndrome**

HAROLD RIFKIN and DOROTHEA ZUCKER-FRANKLIN (by invitation), Montefiore Hospital, New York, N.Y.

**Quantitative Studies of the Effect of Conventional Therapy upon Glucose Disposal in Diabetes Mellitus**

J. A. MOORHOUSE and N. J. ROSEN (by invitation), University of Manitoba and the Winnipeg General Hospital, Winnipeg, Manitoba, Canada

**A Preliminary Report on a New Oral Hypoglycemic Agent (Urea,1-(hexahydro-1-azepinyl)-3-p-tolylsulfonyl-)**

WILLIAM A. ABELOVE, RAUL ECHENIQUE (by invitation), and A. GORMAN HILLS, University of Miami School of Medicine, Miami, Fla.

## BY TITLE

**Candy Tolerance Test for Diabetes Detection**

FRANK N. ALLAN and LLOYD W. GEORGESON (by invitation), Lahey Clinic, Boston, Mass.

**The Significance of the Occasional Success in Treatment of Diabetic Children with Oral Therapy Exclusively**

HENRY DOLGER, The Mount Sinai Hospital, New York, N.Y.

**Evaluation of Phenformin Therapy in Stabile and Labile Diabetes**

GEORGINA FALUDI, PHILIP GERBER, ZEV W. CHAYES (by invitation), and LEWIS C. MILLS, Hahnemann Medical College and Hospital, Philadelphia, Pa.

**Lipoprotein Studies in Lipoid Proteinosis: A Prediabetic Lesion?**

A. E. FREEDLENDER (by invitation), M. D. PHELPS (by invitation), R. ECCLES (by invitation), S. B. REES (by invitation), and R. CAMERINI-DAVALOS, Diabetes Foundation, Inc., Lahey Clinic, Harvard Medical School and Harvard School of Public Health, Boston, Mass.

**Reserpine Action on Carbohydrate Metabolism: Prolonged Glucose Disappearance Rate, Hyperglycemia and Hypoglycemia**

EDUARDO GAITAN (by invitation), and MATILDE MIZRACHI (by invitation), University del Valle, School of Medicine and University Hospital, Cali, Colombia, S. A.

**Liver Function in Forty-four Patients During Chlorpropamide Therapy**

E. A. HAUNZ, W. E. CORNATZER (by invitation), MILTZA LUPER (by invitation), and MARLENE AUBOL (by invitation), Guy and Bertha Ireland Research Laboratory, University of North Dakota School of Medicine and Grand Forks Clinic, Grand Forks, N. Dak.

**Insulin Antibody Levels in the Sera of Diabetic Patients in Relation to the Occurrence of Nonatheromatous Vascular Disease**

Y. HIRATA, SIDNEY GOLDENBERG, N. W. DREY, B. TAUSSIG (by invitation), and HERMAN T. BLUMENTHAL (by invitation), The Jewish Hospital, St. Louis, Mo.

**A Comparative Study in Rat Diaphragm and Fat of Insulin Inhibitory Factors**

HARRY KEEN (by invitation), and JAMES B. FIELD, National Institutes of Health, Bethesda, Md.

**Latent Diabetes Due to Protection of β Cells Against Alloxan Toxicity by Cortisone Pretreatment**

SYDNEY S. LAZARUS and BRUNO W. VOLK, Isaac Albert Research Institute of the Jewish Chronic Disease Hospital, Brooklyn, N. Y.

**Ketoacidosis Induced by Pregnancy in Renal Glucosuria with Aminoaciduria**

ALFRED R. LENZNER, CHARLES F. BISHOP (by invitation), GEORGE F. KOEPF, and AVERY SANDBERG (by invitation), Buffalo General Hospital and the Medical Foundation of Buffalo, Buffalo, N. Y.

**A Portable Automatic Instrument for the Rapid Determination of Blood Glucose Concentration**

JACK R. LEONARDS, Western Reserve University School of Medicine, Cleveland, Ohio

**Treatment of Unstable Diabetes**

ALEXANDER MARBLE, Joslin Clinic and New England Deaconess Hospital, Boston, Mass.

**Simultaneous Changes in Hepatic Glucose Production, Hepatic Glucose Utilization and Hepatic Glucose Balance During Glucose Loading**

D. M. MEBANE (by invitation), F. R. LECOCQ (by invitation), and LEONARD L. MADISON, University of Texas Southwestern Medical School, Dallas, Tex.

**Metabolism of Fructose and Glucose in Vitro in the Normal and Diabetic Rat Diaphragm**

TAKESHI MORIWAKI (by invitation), and BERNARD R. LANDAU, Western Reserve University School of Medicine, Cleveland, Ohio

**Experience with Intravenous Tolbutamide Tests in Individuals with a Family History of Diabetes**

WILLIAM W. H. POTE, Jr., BURT COCHRAN, Jr., and RICHARD BROWN (by invitation), College of Medical Evangelists, Los Angeles, Calif.

**Improvement of Glucose Tolerance in Adult-onset Diabetics on High Carbohydrate—Low Fat Diets**

JOHN E. POTTS, Medical Research Institute, Blalock Memorial Foundation, Walla Walla, Wash.

**Clinical Significance of the Two-hour Postprandial Blood Glucose in Insulin-dependent Diabetes**

DONALD A. ROCKWELL (by invitation), KELLY M. WEST and J. A. WULFF (by invitation), University of Oklahoma Medical Center, Oklahoma City, Okla.

**The Hypoglycemic Activity of Nicotinic Acid in Rats**

MARY A. ROOT and JAMES ASHMORE, The Lilly Research Laboratories and Indiana University School of Medicine, Indianapolis, Ind.

**The Relationship Between Blood Sugar and Serum Osmolality in Maturity-onset Diabetes Mellitus**

DAVID L. SINGER (by invitation), and NORBERT FREINKEL, Harvard Medical School and the Howard Hughes Medical Institute, Boston, Mass.

**Uptake of Free Fatty Acids by the Liver in Normal and Diabetic Dogs**

JOHN J. SPITZER (by invitation), and WILLIAM T. McELROY, Jr., Hahnemann Medical College and Hospital, Philadelphia, Pa.

**An Analysis of 14,888 Individuals Screened for Diabetes in New York City (1957-1959)**

IRVING STARIN (by invitation), EDWARD BADER and KERMIT E. OSSERMAN, The Mount Sinai Hospital, New York City Department of Health, Jewish Memorial Hospital and Albert Einstein College of Medicine, New York City and Bronx, N. Y.

**The Application of Fluid Balance Principles in the Treatment of Diabetic Acidosis**

WILLIAM J. STEENROD, Jr., JOSEPH H. CRAMPTON and ROBERT L. REEVES, The Mason Clinic, Seattle, Wash.

**Plasma 17-Hydroxycorticosteroids in the Pregnant Diabetic**

EDWARD TOLSTOI, WILLIAM P. GIVEN (by invitation), ROY W. BONSNES (by invitation), and AMY F. MARNEY (by invitation), New York Hospital Cornell University Medical College, New York, N. Y.

**Defective Carbohydrate Metabolism in the Cortisone-treated Patient with Adrenal Insufficiency**

BERNARDO LEE WAJCHENBERG, V. G. PEREIRA (by invitation), J. SCHNAIDER (by invitation), A. GELMAN (by invitation), EMILIO MATTAR (by invitation), and A. B. de ULHOA CINTRA (by invitation), Hospital das Clinicas and University of Sao Paulo Medical School, São Paulo, Brazil

**The Futility of Chasing Rainbows**

DAVID H. WALWORTH, Santa Clara County Hospital, San Jose, Calif.

**Clinical Experience with Glucagon**

FRED W. WHITEHOUSE and WILLIAM L. LOWRIE, Henry Ford Hospital, Detroit, Mich.

**The Response to Inflammation in the Diabetic**

FRED W. WHITEHOUSE and JOHN REBUCK (by invitation), Henry Ford Hospital, Detroit, Mich.

**Coronary Blood Flow in Diabetes**

J. A. WULFF (by invitation), KELLY M. WEST, and D. PATRICK (by invitation), University of Oklahoma Medical Center, Oklahoma City, Okla.

## DATE CHANGE FOR 1962 ANNUAL MEETING

June 23-24, 1962, is the date of the Twenty-second Annual Meeting of the American Diabetes Association which will be held in Chicago at the Conrad Hilton Hotel. The date has been changed from that previously announced (June 9-10) since the American Medical Association has rescheduled its annual session to the week beginning June 25.

## POSTGRADUATE COURSE SERIES

The American Diabetes Association will hold its Tenth Postgraduate Course in Detroit, Michigan, Jan. 17, 18 and 19, 1962. Further information on the Course will be published in forthcoming issues of the Journal.

The NINTH POSTGRADUATE COURSE offered by the American Diabetes Association was held in New Orleans, Louisiana, Jan. 18, 19 and 20, 1961. Developed by the Committee on Professional Education of the American Diabetes Association in cooperation with the Schools of Medicine of Louisiana State University and Tulane University, the Course had as its director Thaddeus S. Danowski, M.D., Pittsburgh, Committee Chairman. Arthur R. Colwell, Sr., M.D., Chicago, was Vice Chairman of the Committee.

Daniel W. Hayes, M.D., Louisiana State University, Governor of the American Diabetes Association for the State of Louisiana, was Chairman of the Local Committee. The site of the Scientific Program was the Louisiana State University School of Medicine, and the Jung Hotel served as headquarters.

A registration breakdown for the Ninth Postgraduate Course, and a comparison with the eight previous Courses follow:

| REGISTRANTS | 1961 New Orleans | 1960 Los Angeles | 1959 St. Louis | 1958 Atlanta | 1957 Columbus | 1956 Dallas | 1955 Philadelphia | 1954 Rochester | 1953 Toronto |
|---|---|---|---|---|---|---|---|---|---|
| Members | 72 | 61 | 55 | 78 | 83 | 82 | 135 | 83 | 93 |
| Membership applications pending | 11 | 15 | 21 | 47 | 55 | 52 | 23 | 24 | 23 |
| Nonmembers | 51 | 73 | 31 | 53 | 57 | 47 | 18 | 17 | 61 |
| *Total* | 134 | 149 | 107 | 178 | 195 | 181 | 176 | 124 | 177 |
| OTHER REGISTRANTS | | | | | | | | | |
| Part-time registrants | 5 | 3 | 2 | 1 | 1 | — | 2 | — | — |
| *Graduate Students | — | — | — | — | — | — | 1 | † | † |
| *Fellows | 12 | 7 | 7 | 1 | — | 2 | 3 | † | † |
| *Residents | 22 | 19 | 10 | 14 | 16 | 10 | 10 | † | † |
| *Interns | 1 | 6 | 8 | 4 | 8 | 4 | 5 | † | † |
| *Medical Students | † | 1 | 23 | 2 | 13 | 61 | 11 | † | † |
| *Dietitians, Dietetic Interns | — | — | 2 | — | 16 | — | — | — | — |
| *Nutritionists | — | — | — | — | 2 | — | — | — | — |
| *Nurses | — | — | — | — | 7 | — | — | — | — |
| Councilors and Faculty | 35 | 47 | 41 | 40 | 34 | 48 | 41 | 35 | 35 |
| Diabetes Program Directors Workshop Participants | — | 28 | — | — | — | — | — | — | — |
| Insulin Bioassay Conference Participants | — | 4 | — | — | — | — | — | — | — |
| Other Guests | 8 | 9 | 7 | 6 | 12 | 4 | 6 | — | 2 |
| *Total* | 83 | 124 | 100 | 68 | 109 | 129 | 79 | 35 | 37 |
| *Total of all categories* | 217 | 273 | 207 | 246 | 304 | 310 | 255 | 159 | 214 |
| ADDITIONAL DATA | | | | | | | | | |
| Cancellations | 16 | 9 | 8 | 19 | 12 | 12 | 15 | 25 | 5 |
| Applications rejected due to limited facilities | — | — | — | — | — | — | — | 47 | 92 |
| Attendance at Banquet | 193 | 200 | 173 | 245 | 200 | 324 | 246 | 208 | 236 |

*From metropolitan area of Course location.

†Graduate Students, Fellows, Residents, Interns and Medical Students were not formally registered in 1954 and 1953; a few were in attendance. Medical Students were not formally registered in 1961; approximately fifty were in daily attendance.

## RESEARCH FELLOWSHIP AWARDS

The Committee on Research and Fellowships of the American Diabetes Association announces the award, for the 1961-1962 academic year (July 1, 1961—June 30, 1962), of the following Research Fellowships:

*John A. Colwell, M.D.*, Chicago, will continue his work with Arthur R. Colwell, Sr., M.D., in the Department of Medicine, Northwestern University Medical School, on the effects of various substances on insulin secretion in the dog.

*Jack Kane Goldman, M.D.*, Pittsburgh, will continue to work with George W. Thorn, M.D., and Albert E. Renold, M.D., in the Department of Medicine, Harvard University Medical School, Boston, on the metabolism of mannose and glycerol.

*James C. Barrett, M.D.*, Birmingham, will study insulin resistance in man with Dr. S. R. Hill, Jr., at the University of Alabama Medical Center, Birmingham.

*John K. Davidson III, M.D.*, Columbus, Georgia, will work with Reginald E. Haist, M.D., Ph.D., at the Charles H. Best Research Institute, Toronto, Canada, on functional and structural changes in the islets of Langerhans in relation to the formation and secretion of insulin.

*Philip Levy, M.D.*, Chicago, will work with Dr. W. J. H. Butterfield at Guy's Hospital, London, on the effects of diabetes mellitus on cerebral glucose metabolism.

*Jesse Roth, M.D.*, New York, will work with Solomon A. Berson, M.D., at the Veterans Administration Hospital, New York, on insulin sensitivity in early maturity onset diabetes.

### AVAILABILITY OF NEW FELLOWSHIPS

The Committee on Research and Fellowships of the American Diabetes Association will award at least three Research Fellowships for the academic year July 1, 1962—June 30, 1963. Deadline for applications is Nov. 15, 1961. Requests for application forms and other inquiries should be addressed to Mr. J. Richard Connelly, Executive Director, who will forward the information to the Committee.

## 1962 LILLY AWARD

The following stipulations govern the contest for the sixth Lilly Award, which will be given at the Twenty-second Annual Meeting of the American Diabetes Association June 23-24, 1962, in Chicago. The award is supported by Eli Lilly and Company and consists of $1,000 and a medal.

*Purpose*: To recognize demonstrated research in the field of diabetes, taking into consideration independence of thought and originality.

*Eligibility*: Any investigator in an appropriate field of work closely related to diabetes who is less than forty years of age on January 1 of the year in which the award is made. The research will not necessarily be judged in comparison to the work of more mature and experienced workers. The candidate should be a resident of the United States or Canada.

*Nominations*: Nominations for the award will be solicited from the members of the American Diabetes Association. Such nominations will be requested by repeated notices to be published in DABETES. Names of nominees will be sent to the Chairman of the Committee on Scientific Awards and must be received before December 1 of the year preceding the award. The nomination should be accompanied by full information concerning the nominee's personality, training and research work. Six copies of each item should be submitted. No member may send in more than one nomination. A list of the nominee's publications, if any, and six copies of the publication or manuscript for which the award is to be given should also accompany the nomination. At the discretion of the Committee on Scientific Awards, the award may be given for work published during the year prior to December 1 of the year preceding the award. The nominee should be actively engaged at that time in the line of research for which the award is to be made.

*Announcement*: The name of the winner will be announced in the program of the Annual Meeting of the Association, and the award presented at that meeting. The winner, subject to the approval of the Committee on Scientific Programs, will be invited to present a paper on his work. Papers considered for the award must be submitted with the idea that they will be published in whole or in part in DIABETES if found acceptable to the Editor and/or Editorial Board. If the Committee should decide that no outstanding work has been presented for this consideration, the award will not be made.

*Award*: In addition to the monetary award and medal, traveling expenses will be given to make it possible for the recipient to receive his award in person at the Annual Meeting.

## NAME DELEGATES TO 4th IDF CONGRESS

Francis D. W. Lukens, M.D., Philadelphia, and J. Richard Connelly, New York, have been named Physician and Lay Delegates of the American Diabetes Association to the Fourth Congress of the International Diabetes Federation in Geneva, Switzerland, July 10-14, 1961. The preliminary program for the Congress was published in the January-February issue of the Journal.

## ENDOCRINE GLAND SERIES IN ADA *FORECAST*

In response to many requests, an article dealing with the endocrine glands and their functions appears in the May-June 1961 issue of FORECAST. It is the first of a three-part series by George J. Hamwi, M.D., of Columbus, Ohio. A full-page illustration in color clearly reveals the anatomical location of the endocrines, and their structure and function are explained in the text in understandable language. Copies of the May-June issue may be obtained at 35¢ each or six for $2.00.

## NEW MEMBERSHIP DIRECTORY

The names of all members of record as of March 1, 1961, will be published in an up-to-date Membership Directory of the American Diabetes Association. The Directory will include an alphabetical and geographical listing of Active Members, an alphabetical listing of Associate and Corporate Members, and other information of organizational interest. It will be available later in the year and will be sent to all members without charge.

## ADA SCIENTIFIC EXHIBIT ON DISPLAY

"Everyday Problems in Diabetes: 201 Crystal-clear Answers," the latest scientific exhibit of the American Diabetes Association, will be displayed in New York City at the annual meet-

ing of the American Medical Association June 25-30, 1961, in Space No. 2334, 3rd Floor, Scientific Exhibit Section, New York Coliseum.

This exhibit was developed by the ADA Committee on Scientific Exhibits last year: Marshall I. Hewitt, M.D., Chairman; DeWitt E. DeLawter, M.D.; Alfred H. Free, Ph.D.; Robert C. Hardin, M.D.; William R. Kirtley, M.D.; Leo P. Krall, M.D.; C. J. O'Donovan, M.D.; George F. Schmitt, M.D., in collaboration with the Steering Committee of the Committee on Professional Education as follows: T. S. Danowski, M.D., Chairman; Arthur R. Colwell, Sr., M.D., Vice Chairman; Jerome W. Conn, M.D., Vice Chairman. Dr. O'Donovan has since become Chairman of this Committee, succeeding Dr. Hewitt who continues as Vice Chairman.

Following its display at the AMA meeting, this exhibit will be added to the scientific exhibits already available on a loan basis to Clinical Societies of Affiliate Associations and to other medical organizations. These exhibits are as follows: "Diabetes—Today and Tomorrow: The Expanding Role of the Doctor," "Pathology of Diabetes," "Management of Diabetes Mellitus," "Vascular Complications of Diabetes," and "Diabetes Detection by the Physician."

## NOVEMBER 12-18 IS DIABETES WEEK

Affiliate Associations and Committees on Diabetes of County and State Medical Societies are urged to begin planning their Public Education and Detection programs for Diabetes Week, November 12-18. Diabetes Week is observed the week before Thanksgiving.

## *DIABETES* BINDERS AVAILABLE

Sturdy binders for your 1961 issues of DIABETES are now available at $2.25 each. Binders for prior years also are available at the same price.

A full set of binders for all ten volumes (1952-1961 issues) is offered at a package price of $20.00.

Each of the attractive binders will hold a year's issues of DIABETES and the annual index supplement. Of simulated dark blue leather with gold lettering, they will grace your library shelf and add years of life to your copies of DIABETES. Address your orders now for immediate shipment to: American Diabetes Association, Inc., 1 East 45th Street, New York 17, N. Y.

## NEW MEMBERS

The following were elected as of April 1, 1961:

Active

| | |
|---|---|
| *Alabama* | |
| Porter, Charles E. | Fairfield |
| Selikoff, Solomon J. | Montgomery |
| *California* | |
| Berk, Morris | Oakland |
| *Georgia* | |
| Fitzpatrick, Paul E. | Dalton |
| *Indiana* | |
| Chiu, Gin-Chi | Indianapolis |
| *Iowa* | |
| Hertko, Edward J. | Des Moines |
| *Louisiana* | |
| Gordon, Douglas L. | Baton Rouge |
| *Maryland* | |
| McDonald, Glen W. | Bethesda |
| *Massachusetts* | |
| Contompasis, Peter H. | Winchester |
| Gillespie, Luke | Boston |
| Schonfeld, Arnold | Boston |
| *Missouri* | |
| Auner, Cecil Ray | Springfield |
| Hirata, Yukimasa | St. Louis |
| *New York* | |
| Feldman, Elaine B. | Brooklyn |
| Linder, Morton H. | Valhalla |
| Thurber, David C. | Rochester |
| *Ohio* | |
| Dominguez, Jose M. | Cleveland |
| Kreines, Kenneth | Cincinnati |
| Post, Harvey J. | Cleveland |
| *Pennsylvania* | |
| Calderon, Rolando | Philadelphia |
| *Rhode Island* | |
| Pesare, P. Joseph | Providence |
| *Texas* | |
| Dale, Sebron C. | Houston |
| *Utah* | |
| Perkoff, Gerald T. | Salt Lake City |
| *Washington* | |
| Paton, Richard R. | Seattle |
| *Wisconsin* | |
| Blair, A. James, Jr. | Madison |

## *NEWS OF AFFILIATE ASSOCIATIONS*

The DELAWARE VALLEY DIABETES ASSOCIATION, formerly The Philadelphia Metabolic Association, conducted an open forum on Monday, May 8, 1961, at 8:15 p.m., in the auditorium of The Philadelphia County Medical Society, 21st and Spruce Streets, Philadelphia. The Program included "Psychological Aspects of Childhood Diabetes," by William B. Kennedy, M.D.; "Living with Diabetes," by Peter Koch, M.D.; "Movie of Camp Firefly"; "Exhibit of Menus and Recipes for Diabetics," by the Philadelphia Dietetic Association; and a "Question and Answer Period."

The FRESNO COUNTY DIABETES ASSOCIATION will hold its annual Clinical Society meeting on May 16, 1961, at which Leo Goodman, M.D., will speak on "Glucagon." The annual Meeting of the Board of Directors will be May 23.

The MICHIGAN DIABETES ASSOCIATION held its annual meeting on Wednesday, May 3, at 8 p.m. at the McGregor Center in Detroit. The Clinical Society held its annual dinner meeting and election of officers on March 7. A symposium was held during the afternoon in connection with the Michigan Clinical Institute at which Francis D. W. Lukens, M.D., Philadelphia, spoke on "The Oral Hypoglycemic Drugs." Following the dinner meeting Dr. Lukens spoke on "Comments on Ketogenesis."

The NEW JERSEY DIABETES ASSOCIATION in cooperation with The New Jersey State Department of Health, presented

The American Diabetes Association includes in its membership more than 2,500 clinicians, teachers and investigators in the field of diabetes and related metabolic disorders.

Membership is open to all qualified physicians and scientists. It requires only the sponsorship and endorsement of two Active Members of the Association. Members receive, without additional charge, a subscription to DIABETES, a copy of the program of the Annual Meeting which includes abstracts of papers presented and read by title, subscription to a newsletter THE AFFILIATE BUILDER and so forth.

Membership applications and further information can be secured by writing to the National Office of the American Diabetes Association, 1 East 45th Street, New York 17, N. Y.

The Third Annual Spring Symposium on "Kidney and Diabetes" at the Princeton Inn in Princeton on Wednesday, May 3, 1961, 2:00-5:00 p.m. The clinical conference was chaired by Harold J. Jeghers, M.D., Jersey City, and included "Pathology of the Kidney in Diabetes," by James Berkman, M.D., New York; "Clinical and Biochemical Aspects of Diabetic Nephropathy," by Harold Rifkin, M.D., New York; and "Urological Problems Associated with Diabetes," by Perry B. Hudson, M.D., New York. Following an intermission, a panel discussion on "Kidney and Diabetes" was moderated by Dr. Jeghers. Panel members included Drs. Berkman, Rifkin and Hudson. Members of the American Academy of General Practice received three hours credit under category II for attendance at this symposium.

# *NEWS NOTES*

## NEW SCIENTIFIC DIRECTOR OF LIFE INSURANCE MEDICAL RESEARCH FUND

William A. Jeffers, M.D., Philadelphia, has been appointed Scientific Director and Executive Officer of The Life Insurance Medical Research Fund, whose offices have been moved to 1030 East Lancaster Ave., Rosemont, Pa. Dr. Jeffers will continue his work at the University of Pennsylvania School of Medicine.

## PERSONALS

### ADA MEMBERS TAKE PART IN ACP POSTGRADUATE COURSES

The following members of the American Diabetes Association will participate in postgraduate courses of the American College of Physicians this spring:

*Postgraduate Course No. 12,* "Gastroenterology," at the University of Pennsylvania Graduate School of Medicine, May 15-19: CARROLL M. LEEVY, M.D., Jersey City, and I. J. PINCUS, M.D., Los Angeles, both Officers of Instruction. Dr. Leevy will speak on "Clinical Applications of Hepatic Vein Catheterization," and participate in a panel discussion entitled "The Pathophysiology of the Liver." Dr. Pincus will speak on "Fundamental Biochemical Aspects of Pancreatic Enzyme Determinations—Amylase, Lipase, Trypsin, Antitrypsin, Leucine, Aminopeptidase and Antithrombin," "Recent Anti-emetics: Indications and Hazards," and take part in panels on "Pancreatic Disease" and "Abdominal Pain—Measures for Relief."

*Postgraduate Course No. 13,* "Current Aspects of Internal Medicine," at The University of Iowa Department of Medicine, Iowa City, June 19-23. Officers of Instruction will be WILLIAM E. CONNOR, M.D., Iowa City ("Lipid Metabolic Errors in Atherosclerosis"); STEFAN S. FAJANS, M.D., Ann Arbor ("The Early Detection of the Diabetic State," "Diagnosis and Treatment of Hyperadrenocorticism," "Myxedema"); ROBERT C. HARDIN, M.D., Iowa City ("Ocular Complications in Diabetes Mellitus" and "Diagnosis and Treatment of Hyperadrenocorticism"); and DANIEL B. STONE, M.B., Iowa City ("Treatment of Diabetic Acidosis" and "Diagnosis and Treatment of Hyperadrenocorticism").

GEORGE M. KNOWLES, M.D., Hackensack, New Jersey, has been appointed director of medicine of the Hackensack Hospital. He has served as the attending physician in charge of the diabetes clinic since 1930.

KERMIT E. OSSERMAN, M.D., New York, has been re-elected chairman of The Myasthenia Gravis Foundation.

HERBERT POLLACK, M.D., New York, has been named to serve on the Federal Aviation Agency.

CECIL STRIKER, M.D., Cincinnati, will give the Alpha Omega Alpha Oration to the faculty and student body of the University of Nebraska College of Medicine on May 24. His subject will be "Misocainia and Serendipity in the History of Diabetes Mellitus." Dr. Striker also has recently been selected President-elect of the Alumnal Association of the University of Cincinnati College of Medicine, and is the present President of the Ohio Academy of Medical History.

## NECROLOGY

ALFRED E. KOEHLER, Santa Barbara, California, born August 31, 1896.

WILLIAM LEVISON, South Orange, New Jersey, born April 12, 1909.

RICHARD M. MCKEAN, Detroit, Michigan, born March 6, 1896.